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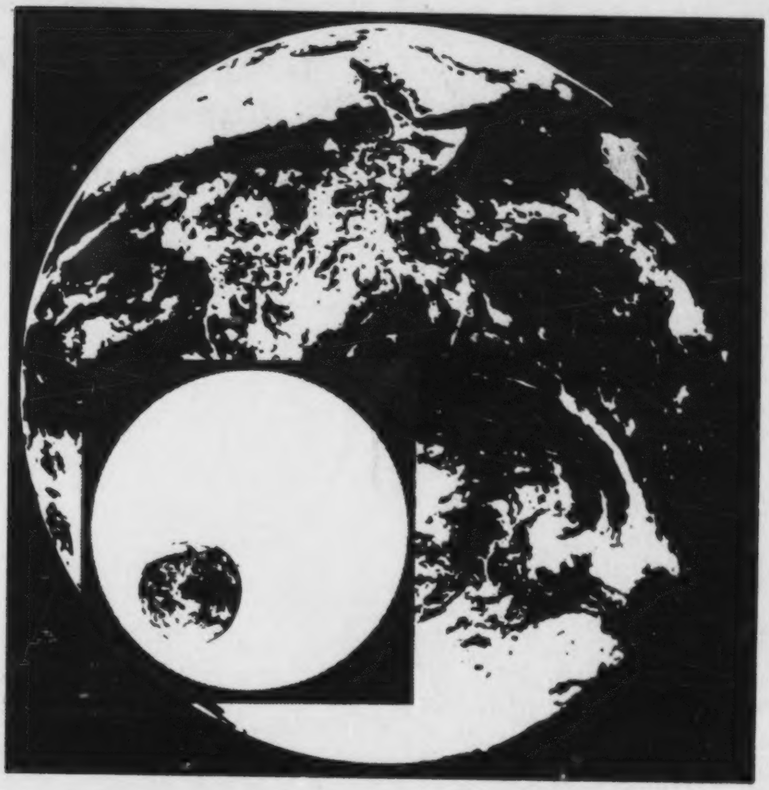
Terrestrial Microcosms and Environmental Chemistry



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TERRESTRIAL MICROCOSMS and ENVIRONMENTAL CHEMISTRY

The Proceedings of two Colloquia, held June 13-14, 1977 at Oregon State University, Corvallis, Oregon as a part of the Symposium on Terrestrial Microcosms and Environmental Chemistry

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Foreword

We take pleasure in dedicating this work to our mentor—Dr. William M. Hoskins, Professor Emeritus of Entomology at the University of California, Berkeley—who understood what we have to learn and passed it on.

These proceedings are the technical papers from two colloquia presented as a part of the Symposium on Terrestrial Microcosms and Environmental Chemistry. Although highly representative of these fields, these papers are not intended to be reviews or syntheses *per se*. Rather, they are basically the research outputs from two particular programs, the NSF-RANN Environmental Benchmark Program and the USEPA Terrestrial Microcosm Project, and from allied studies.

The Symposium, in addition to the two days of public meetings at Corvallis, included a three-day *Workshop on Terrestrial Microcosms*, held immediately after these colloquia, at the Inn at Otter Crest, Otter Rock, OR. This somewhat unusual structure was designed to achieve three objectives:

(a) review the progress in terrestrial microcosm technology, assess the state-of-the-art, suggest a provisional protocol for the use of microcosms in chemical evaluation, and determine the research needs to improve or develop that protocol:

(b) bring together principal researchers in the Environmental Benchmark program for intramural review and interactions with microcosm researchers; and

(c) inform the scientific community about, and receive peer criticism concerning, the current and future research in methodologies designed for evaluation of chemical fate and effects.

The original ideas for the Symposium were more modest. Drs. A. S. Lefohn and N. R. Glass, the originators of the ecological studies under the Substitute Chemicals Program, urged peer criticism to hasten the development of applied terrestrial microcosms. So a small meeting was planned for late in 1976, at which point it was anticipated that sufficient research would be available for a profitable review. Simultaneously the NSF-RANN Environmental Benchmark Program had taken shape and was in need of interaction between investigators. The situation was further stimulated by the successful NSF-RANN/TIE *Workshop on Ecosystem Processes and Organic Contaminents* at Asilomar in 1975. As often happens, several of the persons most desirable as critics of terrestrial microcosm technology were those also involved in various aspects of environmental benchmark evaluations. The result was the typical, rather natural progression from a small joint meeting to the full Symposium.

We felt we were most fortunate in several regards. To the best of our knowledge we were able to bring together all of the laboratories employing terrestrial microcosms to study various aspects of the fate and effects of chemicals in the environment. We had excellent participation from numerous laboratories and the several scientists involved in both the NSF-RANN and USEPA (Office of Pesticide Programs) environmental benchmark research programs. The cooperation between the sponsoring and participating Agencies and institutions maximized the opportunity for synergism. Many of the persons being asked to review microcosm systems had no direct experience with them; some of the microcosm researchers wondered about the applicability of the benchmark approach to what they were doing. Hence, there was a measure of apprehension brought to the Symposium. The participants, however, solved that difficulty with their perceptiveness, kindness, and professional character. We all learned something new.

There were regrets that it was necessary to limit the breadth of the Symposium to terrestrial microcosm systems, as we also learned from attendees at the colloquia and participants in the Workshop who are attacking parallel, but different problems in the area of aquatic microcosm technology. Considerably greater depth could have been added by examining progress in terrestrial microcosms generally, without consideration of chemical insult. An examination of biochemical and toxicological benchmarks may also be warranted. In order to cope with the immediate problems of terrestrial microcosm technology and the benchmark program, however, it was necessary to draw a line. Some of the missing aspects have been addressed elsewhere or will be in the near future.

As with all emerging technologies, microcosm research has its moments of frustration and exhilaration. So it has been with this Symposium. The most stimulating and rewarding aspects have come from the personal interactions with colleagues old and new, exploring and evaluating alternatives in hazard assessment. We hope that this volume (and the separate report on the Workshop) will transmit the essence of those interactions.

Corvallis, OR.

James M. Witt
James W. Gillett

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An Introduction to the Symposium

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Each of us comes to this Symposium with different backgrounds, training, and experiences and with different perceptions and levels of familiarity with microcosms and environmental chemistry. It might, therefore, be useful to review the purposes of this Symposium, the overall issue of prediction of the behavior of chemicals, and the problems of regulation of chemicals.

One purpose is to exposit the research work done in the areas of benchmark chemistry and terrestrial microcosms in order to familiarize each group of research workers with the work in the corresponding area. This will clearly identify where we are now with regard to investigative methods, experimental approaches, utility of the product and interpretations that can be made—in other words, identify the state of the art.

Another purpose is to illuminate the relationships between different types of work. This includes the relationship between the physical chemistry approach, i.e., the environmental chemistry or benchmark program, and the microcosm approach as well as the relationships between microcosms of different designs and different levels of complexity.

A third purpose is to identify how we may begin to conceive of one researcher's products on the basis for design and decision-making in planning another researcher's work. In an integrated effort, the output becomes the input. This is, of course, common to all research, but is not usually a result of conscious and deliberate effort between different fields of research. A deliberate evaluation of input/output relationships is particularly important because you as a researcher can see how someone else becomes a user of your output and may then be motivated to modify your research slightly in order to make it considerably more useful. On the other hand you, as a user, may identify that another researcher is capable of producing a slightly different type of information or product which would enhance your own efforts.

A fourth purpose, then, is to identify how we can construct feed-back loops between these two types of efforts which will enhance the product of both communities in a way that is more effective and more rapid than the usual communication between scientists by means of the published literature or *ad hoc* personal communication.

Finally, we have an ultimate purpose of developing a provisional protocol applying chemical benchmark research and terrestrial microcosm research to the prediction of the fate and effects of both industrial and pesticide chemicals in the environment. As the Symposium proceeds we shall discover by working together the degree to which we can specify protocols, identify problems in research and delineate the kinds and utility of results to be expected from different procedures.

There is a long list of sub-objectives and problems which could be identified but to attempt to do so now would not only take too long but would preempt discussions which we anticipate will be generated throughout this Symposium. There are a number of concepts and questions which we might consider to assist in an overview.

We might consider why we want to predict the behavior, fate, and effects of chemicals in the environment. There are, of course, the obvious scientific reasons of wanting to understand better the behavior of chemicals and the environment, but a succinct and sufficient reason is that the law requires it. One effect of three different legislative acts has been to create a need for identifying the environmental behavior and effects of chemicals for which actual detailed testing has not been conducted—in other words, predicting their behavior. A fundamental purpose of this Symposium is to identify what kinds of data are an acceptable basis for making such predictions. The laws which created the need for the ability to predict chemical behavior are FEPCA/FIFRA, TSCA and RCRA. FEPCA is the Federal Environmental Pesticide Control Act, or the 1972 amendment to FIFRA, the Federal Insecticide, Fungicide and Rodenticide Act; TSCA is the Toxic Substances Control Act of 1976; and RCRA is the Resource Conservation and Recovery Act of 1976, an amendment to the Solid Waste Disposal Act.

These acts are amongst the legislation which is fundamental to our need to be able to predict chemical behavior. Perhaps I shouldn't say "need." We have had the need for more than 50 years, but it was not formally recognized until perhaps 15 or 20 years ago. It now is recognized and required by law. The need to predict behavior and effects in the environment in order to avoid catastrophic episodes in the future poses a very difficult problem which challenges both our science and our resources. When such a desirable act becomes an act required by law it places all manner of additional burdens on the commercial companies producing the chemical, on researchers and science establishment generally, and on the regulatory system in the implementation of the requirements. FEPCA or FIFRA amended is only about 5 years old. In this period of time the USEPA has been evaluating and regulating only a few hundred chemicals. This work load is often referred to as 40,000 pesticides, but this is misleading in that it refers to the wide variety of different formulations. The actual environmental evaluation has been performed on only a small fraction of the total number of active chemicals—the number chosen would depend on the frame of reference. This number is small only in relation to the task being faced now under TSCA and RCRA: evaluating the environmental safety of 30,000 existing industrial chemicals, with perhaps 1,000 new chemicals coming onto the market each year. If the USEPA has been overburdened with the evaluation of the relative few number of chemicals—pesticides regulated under FIFRA, which have an extensive body of environmental behavior data—the question arises: How in the world are we going to do it in a similar manner for all the industrial chemicals? It is impossible to carry out for all chemicals the level of testing required for pesticides. It is questionable whether the pesticide methods are the best or most cost effective method of meeting the goals. For example, does the LD-50 values required for ducks and quail tell us more about the hazard to wildlife in general, or birds in particular, than does the LD-50 values for the domestic chicken? Does trout or bluegill data tell us any more than the values for goldfish? These are questions which I hope this group will consider.

Because of the enormity of the task facing us and the scarcity of resources in terms of laboratories, scientists, time and money, any proposal to obtain data about the fate and effects of chemicals in the environment will have to be most carefully defined. It must be defined with regard to what information we need, why we need it, how it will be used, and what we will be able to predict from it. Any proposal for obtaining data on fate and effects in the environment requires that the process be carefully justified as to scientific validity, its usefulness, whether it answers the problem or not, whether it is practicably achievable, and whether the requirements will be paralyzing to the industry or to society. At the same time the degree of certainty of a prediction and, therefore, its utility must be resolved. The degree of certainty required has grave social consequences. If we wait until the hazard of a chemical has been proved beyond a peradventure of a doubt, then all the birds will be already dead; if we ban a chemical at the first suggestion of a risk, then few or no chemicals will ever be available. Thus there is a social function to determine the acceptability of a risk and the certainty required in prediction. If less than 100% certainty is utilized, then a proposition predicting environmental risk must rest on logic which is demonstrated, understood, and supported by all of those involved in the process. If the logic is not accepted by the regulatee, then you will be constantly answering the question, "What dead birds? I never saw a dead bird!."

The prediction of toxic effects of chemicals has been done for many decades. For most of this time it was largely related to predicting effects on the albino rat and the albino mouse. These were the microcosms of man. As time and understanding progressed, the rabbit, guinea pig, dog, chicken, and finally the monkey were introduced as routine components of this microcosm.

The legal phrase used in the regulation of chemicals is that the purpose is "to prevent adverse effects to the environment." If we are to predict effects of chemicals on the environment, we must use models or "microcosms," and the microcosms which will reliably predict these effects on the various ecosystems have not yet been defined. We are now at a critical time when they must be defined—and very soon! It is not likely that any one system will suffice for all types of chemicals, or that any one system or microcosm will suffice as a model for all ecosystems.

There seem to be three or four approaches and several levels of utility. One sequence of testing or modeling is to proceed through (1) a logic screen, (2) chemical benchmarks, (3) laboratory scale microcosms, (4) biome models, and (5) full-scale field testing. By logic screen I mean identification of the production level, nature and scope of use, the escape rate, and the type of contact or exposure, or in other words, simply a gross sorting out of the magnitude of the potential exposure or how much chemical is there. The greater the amount, the more likely there could be an environmental problem. From that one goes to the properties of the chemical or the chemical benchmark approach. Perhaps we should explain the use of the term "benchmark." It is an ambiguous term which kind of grew as a jargon word in the profession. In order to define it, we need to divide it into two parts. *Benchmark characteristics* are the properties and performance of the chemical used as criteria for decision, or components of a complex conceptual model. *Benchmark chemicals* are reference chemicals for which nearly all benchmark characteristics are known and something about the real world environmental fate and effects are known, and these are known by standardized techniques. The benchmark characteristics can be divided into physical and biological reference points. Under physical and physical-chemical characteristics we would include structure, constants such as vapor pressure, rate of vapor loss, solubilities, partition coefficients, adsorption constants, and reactivities such as oxidation, reduction, hydrolysis, and photolysis rates, etc. Certain characteristics of the biological activity of a chemical could be included as benchmark characteristics, such as toxicity, toxicology, metabolism, metabolic rates, mode or site of action, and so forth. The term microcosm is one of the levels of testing, or approaches, which we are here to identify. We might call them any simplified controlled replication or simulation of an ecosystem. It may be a small test tube of a few grams of soil and the associated microorganisms, or a large enclosure with bushes, trees, grass, frogs, crickets, and so forth. But it must be reasonably well defined. Microcosms vary enormously in scope, design, intent, and mass. They vary 10,000-fold in mass, 10,000,000-fold in volume, and 4-fold or more in the number of trophic levels represented. There are many approaches, many levels of approach, and many levels of testing.

We have brought together for the first time scientists from the National Science Foundation Benchmark program and scientists from the U.S. Environmental Protection Agency Terrestrial Microcosm program because we want to know whether we can predict behavior in microcosms from benchmark studies. We would like to be able to at least predict the limited kind of behavior which can be tested in some microcosms. We would like for microcosm researchers to play a role in developing predictive benchmark models so that they could test the model in their own microcosm. We would like modelers, after a model has been tested in a microcosm, to refine their model to obtain more accurate predictions. We would like to learn what kind of data is needed to construct a model and make a prediction. We would like to learn what sort of predictions can be made with a given type, quantity and quality of data. What is the least set of useful data that should be known about all chemicals? What second set of data should be known about chemicals identified from the first set as needing more study and testing? Throughout both the Colloquia and Workshop, which compose this Symposium, we would like not only to clarify some of the problems mentioned above, but also to consider what levels of confidence are associated with predictions made from models with different sets or bases. What is the likelihood of over-prediction or under-prediction of harm? What about problem chemicals—the chemicals which do not and cannot conform to the predictions—the exceptions and eccentricities?

Finally, it is very important that we begin the process of establishing what are the necessary and sufficient data to permit predictions that will ensure an adequate level of environmental protection. We will need to consider whether it is possible to define a system of benchmark characteristics, various levels of microcosms, and predictive models which will meet the criteria needed for the regulation of chemicals. This will tell us whether the end goal of substituting predictions for endless, exhaustive, and repetitive testing of each and every chemical appears to be feasible. If we cannot do this, if we find no one is satisfied with the probable outcome of predictive modeling based on taking certain chemicals through a sequence or series of gates, according to how they meet the criteria, which we now call level$_1$, level$_2$, level$_3$, or level$_{n-1}$ testing, then we are faced with taking all chemicals to level$_n$ testing. This would probably bring chemical technology to either a standstill, or bankruptcy, or maybe both.

PART 1

COLLOQUIUM ON ENVIRONMENTAL CHEMISTRY AND TOXICOLOGY

Overview of the NSF-RANN Benchmark Program

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Approximately 14 or 15 months before the Toxic Substances Control Act was passed and became the law of the land on January 1, 1977, in June of 1975, we at the Foundation began to have discussions with people in the Office of Pesticide Programs, Criteria and Evaluation Branch—Jack Zweig and his colleagues. They were interested in forming something called the "Benchmark Pesticide Program." At that same time we had become interested in whether, in fact, one could prioritize manufactured organic compounds on the basis of their potential or known impact on the biotic community. So, the notion that perhaps the Environmental Protection Agency and the National Science Foundation could engage in a close, cooperative research program on "benchmark" compounds came into being. We have not, to my own personal satisfaction, ever carried that program to the level and at the rate of progress that it should be at this point. We have provided support to people and institutions to address those questions which Dr. Witt just described. But the feasibility of the benchmark idea is not satisfactorily apparent to me. Is the notion of a surrogate chemical for a given group of substances really practical? And if so, what are the limitations?

The past decade of observations on and inquiry into the incidence of all types of chemically-induced human health disorders and the general deterioration of biological life in the environment has indicated that chronic, low-level exposure to hazardous or toxic chemical substances is quite likely the single, major causal factor. If nothing else, the difficulty we have had in attempting to ameliorate these problems demonstrates that our basic understanding of the behavior of these materials in the environment, and specifically in biological systems, is completely inadequate.

Basic, new knowledge is needed. For example, the validity of bioassay tests in lower organisms as they are judged applicable to predicting toxicological responses in man is of great economic importance as well as being scientifically challenging. Millions of dollars will be committed annually to the testing of currently marketed and new chemical substances and mixtures in order to determine their safety for commercial use.

We are all quite familiar with the two features of biological science which are relevant to the toxicological principles of extrapolation from lower life forms to man. First, there is the *continuity* provided by the evolutionary process whereby all forms of life are related to each other as are the leaves, branches, trunk and roots of a tree. Secondly, there is *variation* whereby no two organisms are exactly the same and remarkable differences distinguish even species that are closely related in the evolutionary sense. The current *art* of extrapolation from bacterial or animal toxicological tests depends on the accuracy of judging whether particular responses to chemicals or other stimuli are part of the general structure of "the evolutionary tree" or are manifestations of variation which affect only limited parts of the "tree." These accuracies are not currently sufficient and much more *targeted genetic research* remains to be completed before unequivocal judgments can be made.

I look forward to the presentations scheduled and the discussions which are bound to develop. The whole issue of predicting things in this area is real and extremely important.

Physical Processes

Mathematical Modeling of Toxic Substances in the Environment

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ABSTRACT

Theories and applications of computer simulation models are given for the propagation and effects of toxic substances in the environment. The mathematical models have very practical objectives involving risk/benefit analysis as well as providing feedback into scientific efforts in the field and in laboratories. In cases where large reservoirs and flows of the toxic substances exist in the physical/chemical environment as contrasted with the biological environment, decoupling is justified by using environmental concentrations as inputs to a separate biota model. Pathways in the physical environmental framework include flows from one medium to another as well as flows connecting geographically differentiated subcompartments within each medium. In the biological framework damage functions can be constructed in terms of lethality or other forms of insult. Model logic, inputs and outputs are discussed as well as various mathematical approximations which simplify solutions. Demonstrations of a compartment model are presented using a prototype aquatic ecosystem. Laboratory microcosms may serve well as prototypes for future model validation so that modeling could forge a logical link with environmental chemistry studies. Experimental requirements raised by the model applications are described and the procedures for model validation are outlined. A long term objective is the rational extension of measurements of "benchmark chemicals" to other candidate chemicals not yet introduced.

INTRODUCTION

We are beginning to recognize that our ability to affect natural systems is growing faster than our ability to quantify these impacts. One example of this is the large and growing number of chemical substances that are placed into the earth's environment. Many of us are involuntarily exposed to these substances. Some of the exposures are characterized by risks to health and welfare; however, the risks are difficult to assess because of the incompleteness of the data base on transport, transformation and biological processes. Some of the materials are directly toxic to human beings, whereas others affect nonhuman organisms in various ways. In addition to direct increases in mortality rate, other irreversible biological effects including carcinogenesis (cancer), mutagenesis (transmissible genetic damage), and teratogenesis (birth defects) may be caused by exposure.

Protection of health and the environment from harmful chemicals and mixtures has been mandated as a national policy through the Toxic Substances Control Act of 1976 (Public Law, 1976). This act is intended to protect against "an unreasonable risk of injury to health or the environment" may be brought about by any newly introduced substance that is not already covered by existing Federal statutes.[1] The risks to health and environment must be assessed by the performance of various laboratory tests prior to permission to market any unlisted chemical. A very difficult logical connection must be made between the laboratory test results and the expected behavior of material in the entire environmental system. Biological simulation models take the form of laboratory microcosms that have been proposed to extrapolate effects in the laboratory to natural ecosystems. Presumably the laboratory microcosm contains surrogate components and processes that represent specific origins, flows, transformations and effects in the open environment.

Mathematical simulation models, which are discussed in this paper, abstract these phenomena and represent them in the form of mathematical variables and relationships. It should be emphasized that the use of biological and mathematical models is complementary and not mutually exclusive. The development of both types of models should follow closely any planning to establish "benchmark compounds." Presumably the physical, chemical, and biological properties of the benchmarks are well known with respect to environmental hazard. Through a limited number of variables a mathematical simulation model will permit extending the knowledge of the benchmark compounds to candidate chemicals possessing analogous structures.

Applications of models are necessarily regarded as long-term objectives. The immediate use is to aid in understanding the fundamental relationships between measured properties, laboratory results and the real world. In this manner the model should help provide feedback into field and laboratory efforts by identifying data needs. As the art of modeling matures it should help decision makers by providing environmental profiles of proposed new chemicals in the preregistration assessments. In this connection, cost/benefit or risk/benefit analyses could be carried out prior to the introduction of the new substance to the market. With further refinement of model performance specific inputs to the legislative and rulemaking processes may be possible using the output of models.

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[1] The following materials are excluded from coverage under the Toxic Substances Control Act: fire arms and ammunition (26 USC 4181), food and drugs (21 USC 301), meat and meat products (21 USC 601), eggs and egg products (21 USC 1031), pesticides regulated by FIFRA (7 USC 136), tobacco or tobacco products and any nuclear materials regulated by the Atomic Energy Act (42 USC 2201).

One of the most ambitious applications of these models could be the establishment of a real-time control system that uses model logic with dynamic feedback to restrict the rates of release of materials into the environment. Such a control system would employ key environmental monitoring indicators which would be telemetered into a central data processor. The model logic would then be employed using these real-time data as inputs to project both long-range and immediate consequences of present activities. The model logic would also evaluate alternative futures involving various rates of application or use of the chemical substance.

Various approaches may be taken in constructing mathematical simulation models. One possibility is a statistical/empirical model that uses available field data. This type of model has the advantage of being verified on the basis of actual observations of effects as they relate to rates and distributions of introduction of a material into the environment. For a specific substance, however, a disadvantage of this approach is that the model can only be used after the fact unless the user wishes to risk extrapolation into unknown data regimes. One of the possible applications of a statistical model might be its use as a baseline case representing a benchmark compound. New materials in the same family as the benchmark compound might then be assessed by estimating perturbations about the benchmark behavior using laboratory results to derive influence coefficients.

Another approach is the deterministic model which currently is receiving more attention than the statistical/empirical model. The deterministic model must contain mathematical relationships that permit prediction of the dynamics of material flows through the environment and their effects on ecosystem changes. The time dependent nature of the deterministic model suggests the use of differential equations describing the historical evolution of systems. The use of this type of approach involves posing an initial value problem where the original state of the system is specified and successive states are determined by integration of the differential equations. Subsequent sections of the paper illustrate the structure and operations of deterministic models.

It is likely that no single formulation will serve all purposes. A family of special purpose models will evolve to provide decision makers with a variety of information. Classification of the various schemes may be done by time/space resolution, point source versus area source characteristics, impulsive versus continuous source characteristics, acute versus chronic responses, chemically reactive versus inert substances, or even by domain or medium (air, water, soil, or a particular species of the biota). Successive sections of this paper cover some specific model structure, describe the internal logic, inputs, and outputs of one type of simulation, demonstrate the operation of a compartment model for an aquatic ecosystem under stress, and present concluding remarks on the future development and roles of these techniques.

SOME SPECIFIC MODEL CONCEPTS

Relationship to Measurement Programs

Modeling should be related on three levels to ongoing measurement programs. The first level is fundamental laboratory programs involving physical, chemical and biological measurements of substances. Much of the laboratory information on physical and chemical properties such as that enumerated above deals with equilibrium properties of systems whereas the data demanded by models requires rate information. Measuring the rate in one direction for a process as well as the equilibrium constant permits estimation of the rate of the reverse process. Therefore, it is desirable to have rate measurements in one direction as well as equilibrium constant measurements.

A second level of relationships between modeling and measurement is that involving laboratory microcosm experiments. Because of the high degree of control that is ideally attainable with these systems it should be possible to provide validation information for certain portions of a chemical model for the biota. Interaction between the model development and laboratory experiments is essential to take advantage of this scientific interaction. This will provide an excellent opportunity to measure biological rate coefficients if the microcosm experiments track biomass and energy balances in addition to toxic substances levels.

The third and perhaps the most important interplay between theoretical simulations and measurements requires the model be subjected to evaluation tests using observational data measured in the field. Although the investigator must accept what the natural environment presents to him with regard to external conditions, he has the latitude of choosing the quantities to be measured, how frequently they are measured, how dense a monitoring network will be necessary, and what locations are optimal for such a network. Interaction between model exercises and field observation programs will help optimize the choices in these various areas for any given chemical substance of interest. Conversely, it is essential to subject the model calculation to "real world" data obtained in the field.

A recognized difficulty with all modeling efforts at the outset, is that insufficient data exist to test the model completely. It is precisely this problem that is best addressed by the application of models in the early stages of the research. The model serves as a logical structure for organizing exactly what data are required to provide a system description. With this systematic guide, experiments and measurements can be planned to fill in the gaps that are revealed by the attempt to model. Therefore, despite deficiencies in the information base it is never too soon to begin structuring and designing model exercises in order to provide this linkage between the desire to predict based on a limited body of information and the desire to obtain an optimal set of measurements for describing a complex system.

Flow Charts of Environmental Chemical Models

Frequently the flows and concentrations of a substance in the physical/chemical environment are large compared with those in an ecosystem of interest, and the two systems can be mathematically decoupled. That is to say, the uptake or elimination of a particular chemical material by a biological system may impose negligibly small perturbations of the levels of this substance in air, soil, or water. In the compartment model sense even if one considers large bioaccumulation of a substance the total mass involved may still be small. For example, if the mass of all biota is considered to be 1, that of the soil is approximately 100, the atmosphere, 10,000, and water, 1,000,000 (Haque and Freed, 1974.) Despite bioaccumulation, the DDT mass estimated for marine fish is 600 metric tons and plankton 3 metric tons compared with a peak application rate of $\simeq 10^3$ metric tons per year (SCEP 1970). The estimated holding capacity of the

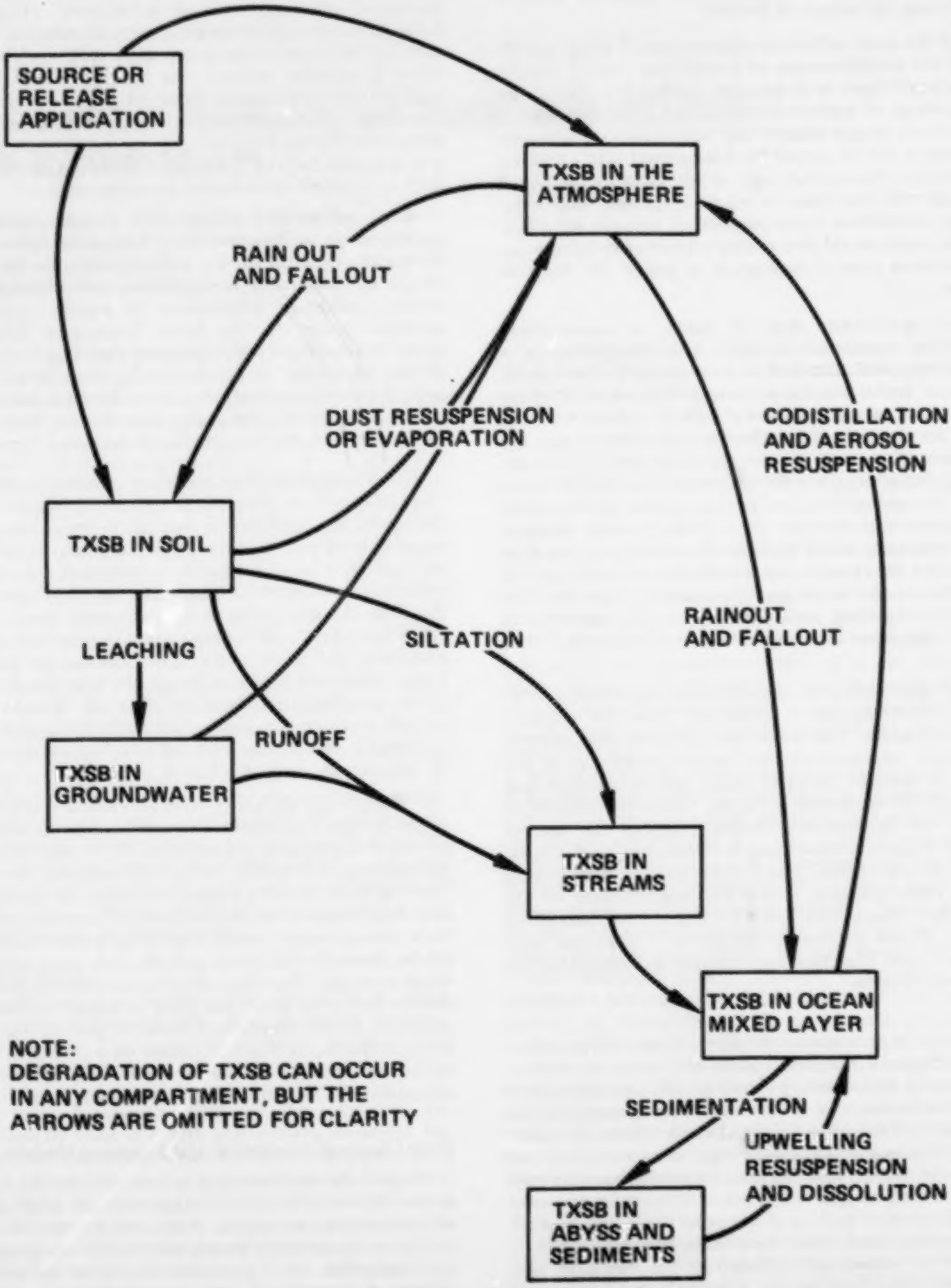

SOURCE OR RELEASE APPLICATION
TXSB IN THE ATMOSPHERE
RAIN OUT AND FALLOUT
DUST RESUSPENSION OR EVAPORATION
CODISTILLATION AND AEROSOL RESUSPENSION
TXSB IN SOIL
LEACHING
SILTATION
RAINOUT AND FALLOUT
RUNOFF
TXSB IN GROUNDWATER
TXSB IN STREAMS
TXSB IN OCEAN MIXED LAYER
NOTE: DEGRADATION OF TXSB CAN OCCUR IN ANY COMPARTMENT, BUT THE ARROWS ARE OMITTED FOR CLARITY
SEDIMENTATION
UPWELLING RESUSPENSION AND DISSOLUTION
TXSB IN ABYSS AND SEDIMENTS

Figure 1. Pathways for toxic substances (TXSB) in the physiochemical environment system.

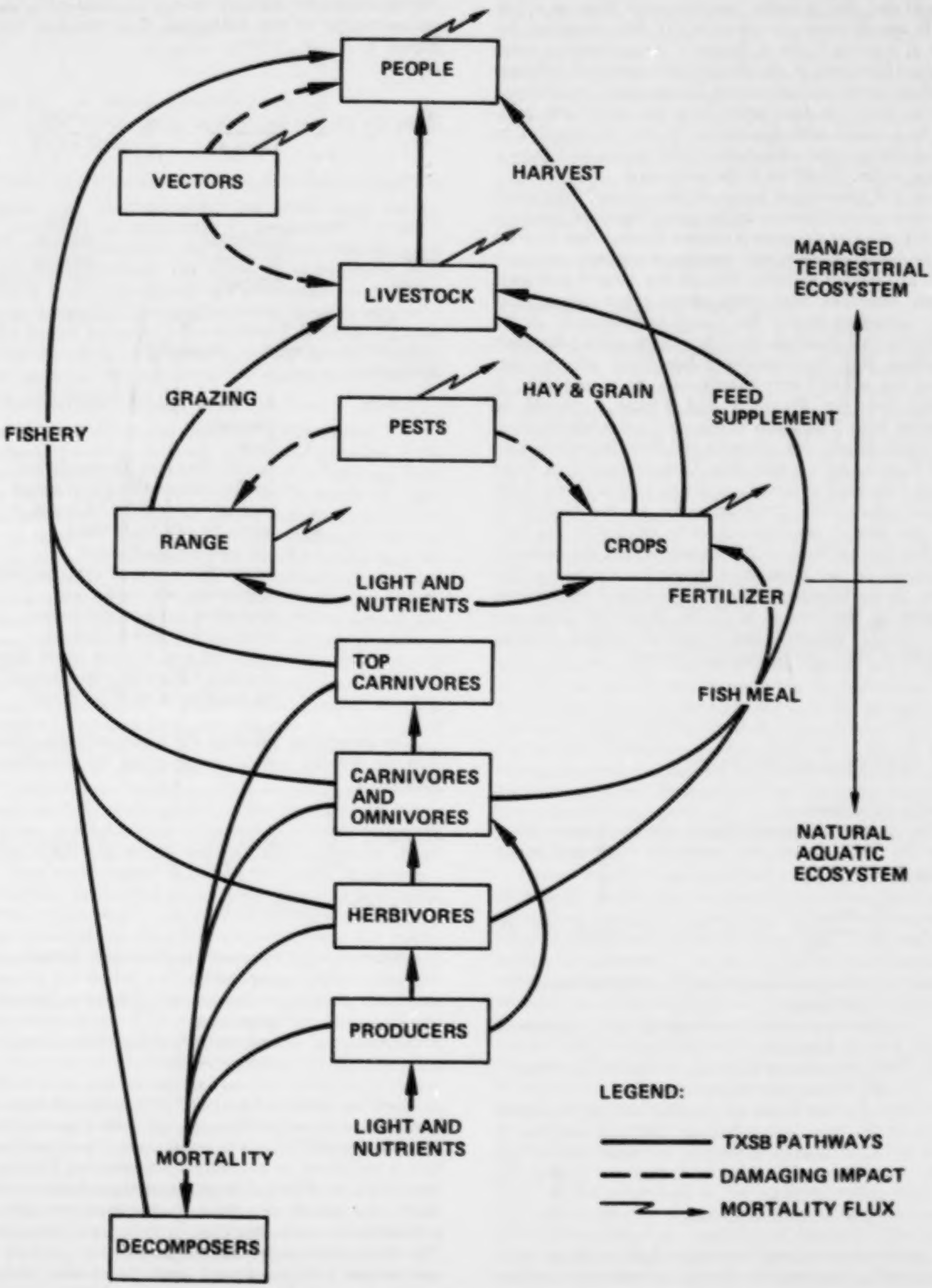

PEOPLE
VECTORS
HARVEST
LIVESTOCK
MANAGED TERRESTRIAL ECOSYSTEM
GRAZING
HAY & GRAIN
FEED SUPPLEMENT
FISHERY
PESTS
RANGE
CROPS
LIGHT AND NUTRIENTS
FERTILIZER
TOP CARNIVORES
FISH MEAL
CARNIVORES AND OMNIVORES
NATURAL AQUATIC ECOSYSTEM
HERBIVORES
PRODUCERS
LEGEND:
LIGHT AND NUTRIENTS
TXSB PATHWAYS
MORTALITY
DAMAGING IMPACT
MORTALITY FLUX
DECOMPOSERS

Figure 2. Pathways for toxic substances (TXSB) in managed and natural ecosystems.

mixed layer (top 100 m) of the ocean is about 10 times the peak annual application rate.

The decoupled systems of the physical/chemical environment and the biological environment may be represented in simple form according to the flow diagrams expressed in Figures 1 and 2. Figure 1 recognizes the commonly used partitions in the physical environment differentiating fresh water and sea water, for example, or differentiating the ocean surface layer from the abyss and sediments. In a real model application it may be possible to eliminate certain compartments or paths shown on Figure 1, depending on the properties of the substance.

Figure 2 differentiates between the natural ecosystem and the managed ecosystem by the use of separate compartments. A particular example is carried through this diagram by the inclusion of pests and vectors as separate compartments. The flows of material through the various pathways indicated, therefore, may bring about either damages or benefits according to the functional specification of the model terms. For example, toxicity effects among the pests and vectors may be considered beneficial whereas influences on other components can be damaging. A difference between Figure 1 and Figure 3 should be emphasized here. The flows in Figure 1 are solely those of the chemical substances, whereas the flow pathways indicated in Figure 2 have a dual role. They indicate both flows of energy expressed as feeding terms as well as flows of the chemical substances. Consequently, in the biological system two sets of equations must be carried in the food unless the toxic effects of the chemical rate are assumed and imposed on the population dynamics equations. The diagrams for model structures are based in part on previous suggestions by Harrison, et al. (1970), Woodwell, Craig and Johnson (1971), Randers and Meadows (1973), Cramer (1973), Gillett, et al. (1974), and Bolin (1976).

LOGIC, INPUTS, AND OUTPUTS

Governing Equations

In the physiocochemical regime species budget equations in the fluid phases are commonly expressed in the following form:

$$\frac{\partial c_j}{\partial t} + \sum_{\ell=1}^{3} \frac{\partial (c_j v_{j\ell})}{\partial \xi_\ell} = w_j + S_j \qquad (j = 1,2,3,\ldots p) \qquad (1)$$

where
c_j = Mass concentration of the jth species
t = time
ℓ = an index referring to each coordinate direction
$v_{j\ell}$ = velocity of jth species in ℓ th direction
ξ_ℓ = distance in ℓ th direction
w_j = net molar production rate of jth species per unit volume by chemical reaction
S_j = source strength for emitters of jth species at some location above the ground
p = number of species

The approach to solving these equations involves varying degrees of approximation. It may be seen that these are nonlinear partial differential equations with the nonlinearities confined to the source and sink terms because the velocity field is given.

In the model structure dealing with the biota, both energy flows and chemical substance flows must be considered. Equation (2) represents one form of a set of governing equations for biomass energy equivalents in each subcompartment of the biological flow diagram shown in Figure 2.

$$\frac{dx_i}{dt} = F_{oi} + \sum_{j=1}^{s} \tau_{ji} \frac{x_j x_i}{(x_j + a_{ji} x_i)} - \sum_{j=1}^{s} \tau_{ij} \frac{x_i x_j}{(x_i + a_{ij} x_j)} - \mu_i x_i$$

Forcing flux — "i" feeding on other species — other species feeding on "i" — first order mortality and excretion

$$-\kappa_{ii} x_i^2 \quad -\lambda_{io} x_i \quad -\rho_{io} x_i \qquad (i=1,2,3,\ldots,5)\ (j\neq 1)\ (2)$$

competitive mortality — export — respiration

where
x_i = biomass energy equivalent of ith population
t = time
F_{oi} = forcing flux into ith population
τ_{ji} = feeding rate coefficient (i eats j)
τ_{ij} = feeding rate coefficient (j eats i)
μ_i = mortality rate coefficient
κ_{ii} = competition coefficient
λ_{io} = emigration or export rate coefficient
ρ_{io} = respiration rate coefficient
a_{ji} = saturation balance parameter
s = total number of populations
j = index of any species other than i (increasing values of index denote ascending position in the food chain)

The governing equation for toxic substance concentration in the ith population is given by Equation (3) as follows:

$$\frac{d(y_i x_i)}{dt} = \sum_{j=1}^{s} \tau_{ji} \frac{x_j x_i y_j}{(x_j + a_{ji} x_i)} - \sum_{j=1}^{s} \tau_{ij} \frac{x_i x_j y_i}{(x_i + a_{ij} x_j)} - \mu_i' x_i y_i \quad (3)$$

$$\kappa_{ii} x_i^2 y_i - \lambda_{io} x_i y_i + w_i$$

where
y_i = concentration of toxic substance in ith population
y_j = concentration of toxic substance in jth population
μ_i' = loss coefficient for toxic substance from ith population

It will be noted in Equation (3) that most of the terms are identical to those in Equation (2) with the simple added feature of multiplying a concentration. For $w_i=0$ this equation is restricted to nonreactive substances. Clearly under the influence of some life processes the substances will undergo changes in the food chain. Equation (3) can be generalized to cover that case by defining a hierarchy of y_i. This hierarchy might be designated as y_{ik} where k is a species index ranging from 1 up to the number of chemical species involved. Thus, those cases would be represented by a coupled set of species mass balance equations for each trophic level.

Lethal toxicity effects might be represented by an expression like that in Equation (4) which imparts values to the mortality coefficient

$$\mu_i = \mu_i^o \left[1 + \frac{\mu_i^{max} - \mu_i^o}{\mu_i^o} \exp(-y_{io}/y_i) \right] \quad (4)$$

where μ_i^o = Baseline mortality rate
μ_i^{max} = Mortality rate at saturation of lethal effects

μ_i that build up from a baseline level μ_i^o to the level μ_i^{max} in accordance with an exponential law based on a characteristic concentration parameter y_{io} expressive of a lethal concentration 50 percent of the way between the extremes and mortality coefficient. For instances of sublethal toxic effects such as an impairment or enhancement or metabolism or mutagenesis, a damage function equation similar to Equation (4) can be constructed knowing the dose-response curves for the effect of interest. In some cases the damage function need not be coupled to the equations if it has no feedback into population dynamics.

Approaches to Solving the Governing Equations

Standard numerical methods of integration using finite difference formulas are widely available in package form and for linear equations are available in terms of user-oriented compilers permitting simple specifications of the system in terms of some intermediate language. Our approach to the solution employs an implicit numerical integration scheme of variable order developed by Gear (1971) suitable for the solution of "stiff" equations. The term stiffness refers to widely differing time constants among the processes represented. Especially with reactive substances this property may be a prominent obstacle to the solution of the equations because of wide variability in reactivity within various trophic levels. Another implicit method which is more forgiving of discontinuous changes is available for solving equations of this class. This method is called EPISODE and was developed by Hindmarsh and Byrne (1975). Both of these techniques are available as working codes and are fully documented so that the user can incorporate them as subroutines to be called without becoming concerned with the internal details of the programs themselves. They are designed to handle nonlinear equations.

Even with the availability of these powerful numerical integration techniques it still may be desirable to incorporate approximations in the governing equations. For example, Equation (1) describing flows in the physical environment can be solved for a system of geographical compartments thereby reducing it to additional ordinary nonlinear differential equations. This coarse resolution representation involves rate coefficients for flow from one compartment to another that are obtained from Equation (1) for transport rates. Finer scale approximations are also available in Equation (1) such as the neglect of transformations by dropping the source terms or the neglect of diffusion in certain directions by dropping components of the diffusive transport terms on the right-hand side.

Typical approximations in the equations for biomass and toxic substance concentration involve dropping spatial resolution entirely, lumping parameters by aggregation of species or levels in the food chain, neglect of competition, neglect of saturation effects in the feeding terms, and linearization of all terms.

As each of these levels of approximation is introduced certain input requirements vanish. With the simple geographical box representation all flow variables except average velocity gradients become unnecessary. Increasing degrees of aggregation of biota within compartments accordingly decreases the requirement for numbers of rate coefficients of initial values for the trophic equations. This is commonly known as a "lumped parameter" approach in the field of simulation. Neglect of competition among species again permits the deletion of further coefficients as does neglect of saturation effects in the feeding terms. Finally, if the instantaneous population effect of acute dose of toxic substance is known *a priori* it can be inserted in the population dynamics description without tracing the flows of the substance through the environment. This will be exemplified in the next section.

COMPARTMENT MODEL DEMONSTRATION

Prototype Ecosystem Description

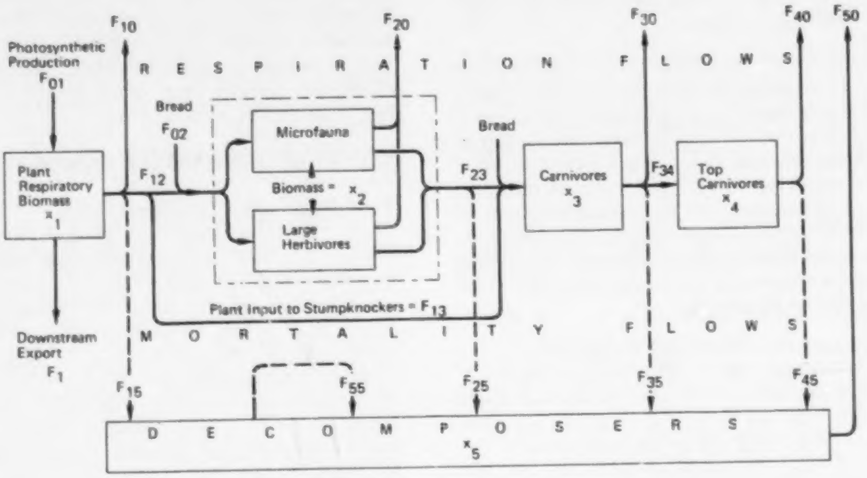
As a point of departure in a demonstration we must choose a prototype ecosystem and specify a model for describing it. It is useful to choose a system which has been measured extensively for the variables of interest. An example approaching satisfaction of this requirement is that established by that of Odum (1957) for the aquatic community in Silver Springs, Florida. Its equilibrium state has been observed to be stable for many years due to a large steady flow of water. External changes lead to periodic diurnal and annual excursions about the mean value of biological parameters. These are averaged out by Odum; coefficients described from these data will restrict the time resolution of the model calculation to the order of a year.

Turning to the specification of a model structure we will employ a box model using coupled ordinary differential equations similar to those outlined in the previous section under Equation (2). The feeding terms have been simplified to omit the saturation effects in the denominators. Energy flows from each population account for changes in the biomass energy equivalent of that population. The state of the system, therefore, is described by a list of biomass energy equivalents, one for each level of the trophic chain. As a starting point for the model we have selected the equation suggested by Patten (1971). From the examples shown in that work we will choose the nonlinear form of the rate equations.

Seven contributions described in the equations are flows of energy across the ecosystem boundary into a given population, feeding of population "i" on other species, mortality of population "i" due to being eaten, spontaneous mortality of population "i" in the first order, spontaneous mortality of population "i" which depends on the square of the ith biomass energy equivalents (competitive), emigration of population "i" across the ecosystem boundary, and net energy loss due to respiration. Figure 3 gives a schematic diagram of the system. According to Odum (1957) the plant respiratory biomass is represented mainly as an algae/sagittaria complex. The herbivore compartment is divided into microfauna such as snails and worms and some of the fish population (mainly Mullets and half of the spotted sunfish or stumpknockers). The carnivores are composed of the other half of the spotted sunfish and catfish. Top carnivores consist mainly of bass and gar pike, whereas the decomposers are crayfish and bacteria. The arbitrary division of sunfish into two compartments is a model method of indicating a branched food chain by assuming equal partition in feeding. The role of competitive spontaneous mortality is represented parametrically and using the equilibrium flows in the system as reported in the literature we derived r_{ij} and the κ_{ij} coefficients assuming some fixed partitioning between first order mortality and competitive mortality.

Modeling Acute Toxic Effects

Acute kill episodes can occur selectively to certain segments of the community depending on toxicity levels. A is simply the injection of a reduced value of the population in question among the initial values that are provided to the population dynamics equation. This further assumes that the killed fish are exported from the ecosystem. The recovery of the system is modeled following the episode. Figure 4 illustrates the recovery of top carnivores after 90 percent of them are killed in a single episode. The various curves show



Photosynthetic Production
F_{01}
Plant Respiratory Biomass
x_1
Downstream Export
F_1
F_{10}
RESPIRATION FLOWS
F_{20}
F_{30}
F_{40}
F_{50}
Bread
F_{02}
F_{12}
Microfauna
Biomass = x_2
Large Herbivores
Bread
F_{23}
Carnivores
x_3
F_{34}
Top Carnivores
x_4
Plant Input to Stumpknockers = F_{13}
MORTALITY FLOWS
F_{15}
F_{55}
F_{25}
F_{35}
F_{45}
DECOMPOSERS
x_5

Figure 3. Schematic diagram of Silver Springs (Florida) ecosystem.

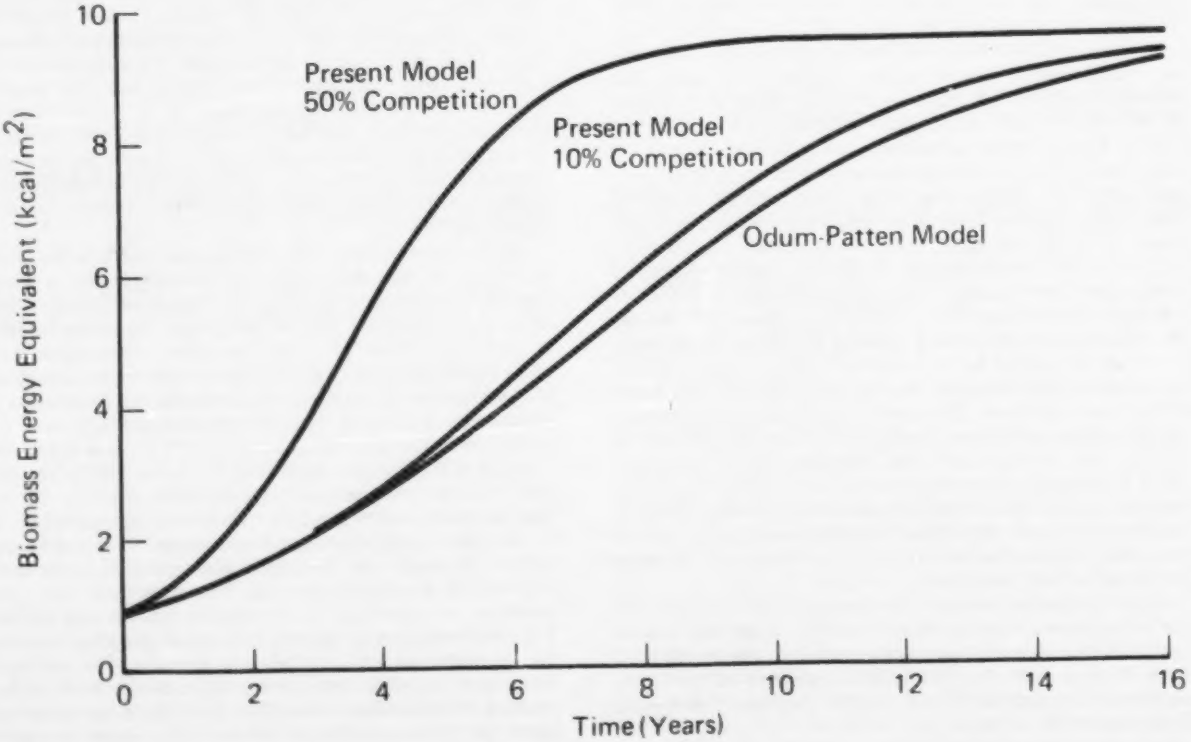


Figure 4. Top carnivores after a 90% kill.

the influence of both chain branching and competition in the population dynamics. The most rapidly returning curve is that characterized by 50 percent competition. The meaning of competition is taken as that percentage of spontaneous mortality due to competitive mortality in the equilibrium system. The system with 10 percent competition appears to take nearly twice as long to recover as that with 50 percent competition. Finally, the Odum-Patten model with no competition and no branching appears to be very closely approximated by the 10 percent competition curve. The strong competitive effect occurs because the second order mortality terms drop off faster than the first-order ones when the population is depressed.

Going deeper into the food chain a 90 percent kill episode was imposed on the herbivore population in another simulation. Figure 5 illustrates the profound effect in this case of chain branching on ecosystem recovery. It should be noted that much of the difference between the carnivore and herbivore curves appears because of the reallocation of stumpknockers to account for their feeding options.

Competitive mortality as well as options in feeding affects the ecosystem recovery from the 90 percent herbivore kill. Figure 6 shows historical plots of the aftermath exhibiting a smaller die-off and a faster restoration for a highly competitive community. These rapid changes may not be accurately represented by the model because of the time resolution problems referred to earlier. While herbivores and carnivores show only small percentage changes in the presence of higher competition the top carnivores show relatively large change. Comparison of the 0 and 90 percent competition cases shows this clearly. For the high competition factor, about half as much die-off is observed compared with the case for low competition. Apparently the recovery time as well as the die-back is reduced by higher competition, whereas chain branching reduces the die-back without much change in recovery time.

Modeling Chronic Toxic Effects

For the study of chronic toxic effects, some preliminary calculations of biomagnification were carried out to test for plausibility.

An initial attempt of adjusting mortality/excretion loss terms (μ'_i) in Equation (3) did not reflect the correct time response to get magnifications typical of those for chlorinated hydrocarbons. The input forcing flux was set at a value of 10^{-12} mass units of toxic material per kilocalorie of energy flow into the producer compartment (this is a completely *ad hoc* figure corresponding to an equivalent absorption of 1 ppt contamination of material with 1 kcal/gm

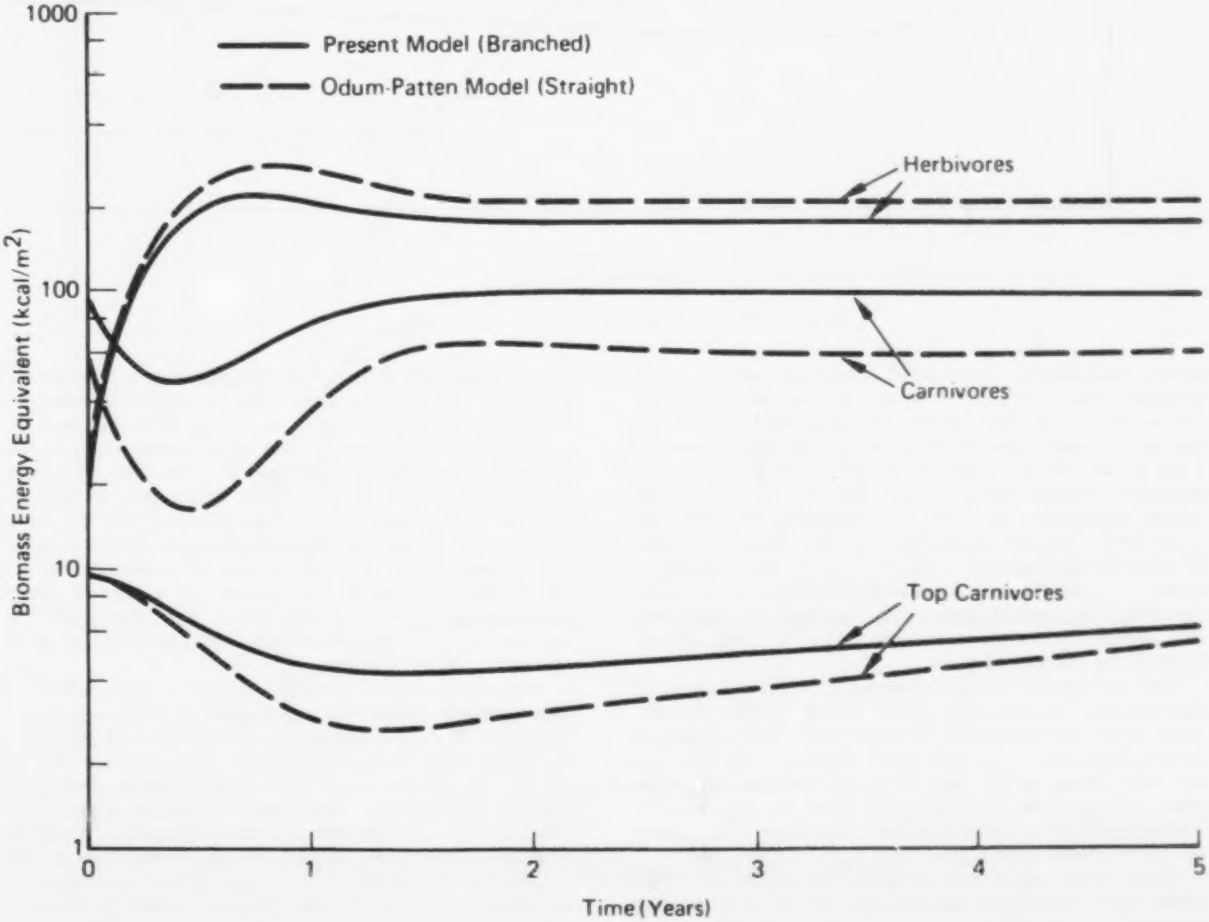


Figure 5. 90% kill of herbivores-chain branching.

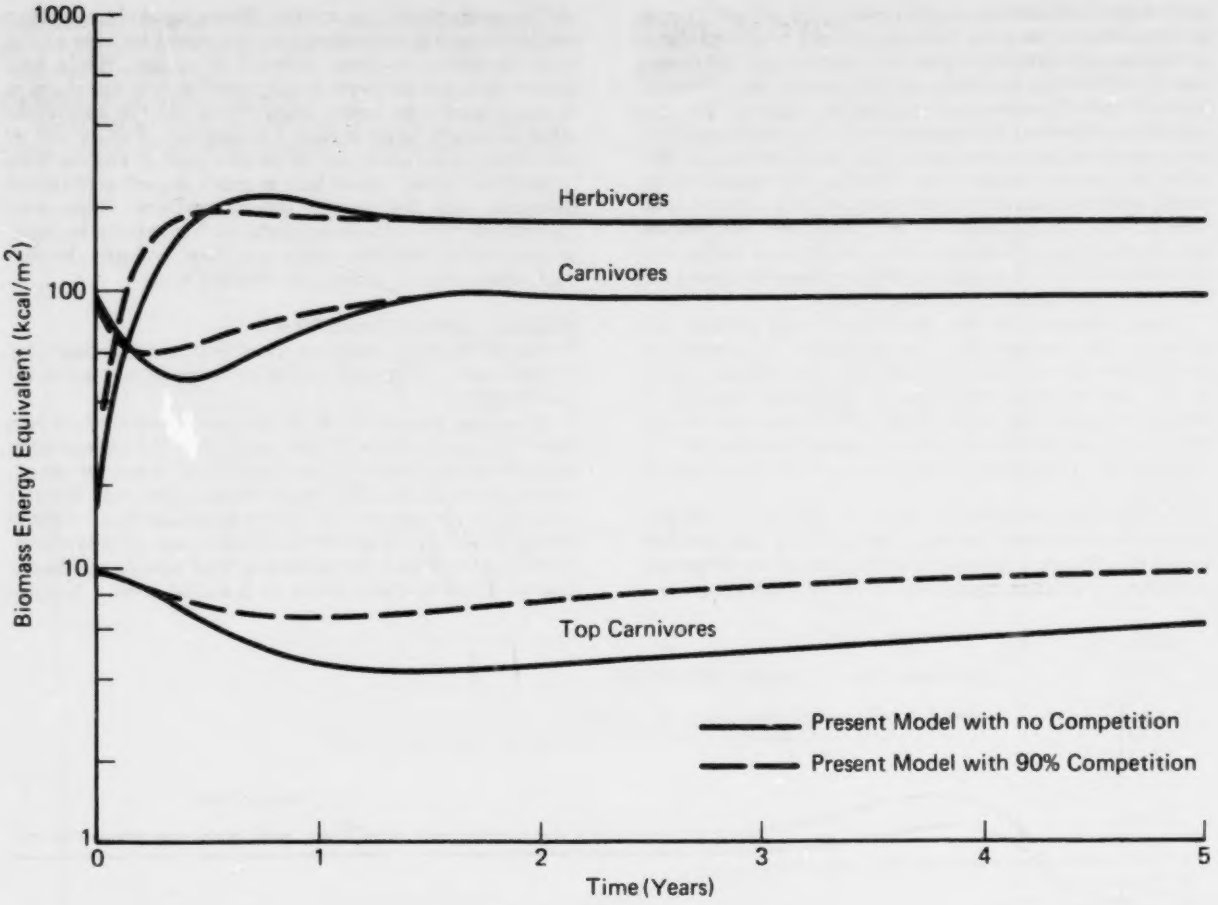


Figure 6. 90% kill of herbivores-Lotka-Volterra competition.

energy equivalent). Downward adjustment of the mortality/excretion coefficients by 10^{-2} for the producers, 10^{-3} for the herbivores, and 10^{-4} for the carnivores, and 10^{-5} for the top carnivores gave equilibrium concentration values of 4 ppt, 30 ppt, 300 ppt, 1 ppm and 10 ppm of material in the five respective compartments. Since the relative values appeared reasonable in light of environmental data for DDT/DDE a dynamic simulation was run with only a trace of contaminant material (1 ppt) initially in each compartment. The results were implausible because after 100 years following the onset of application, the top carnivores were still several orders of magnitude below the equilibrium level of the order of 10 ppm.

The second simulation of biomagnification took a much simpler form than the first one. It was noted that the producer level equilibrates rapidly to a concentration nearly in direct proportion to the input flux, and only that flux was adjusted. Observed DDT levels in plants are typically of the order of magnitude of 10^{-2} ppm; therefore, the flux was increased to place the concentration in that range. This turned out to be 10,000 times higher than with the first trial.

Now, in the second version of the simulation all of the other adjustments were removed and the coefficients were restored to their original values. The flow of contaminant into a level was related through predation coefficients, biomass energy equivalents, and concentration (in mass of contaminant/unit biomass energy equivalent). The loss was similarly related to the prey terms and mortality/excretion flows without any adjustments. As shown in Figure 7, the system response showed a buildup of contaminant in four years to approximately 0.02 ppm in producers, 0.1 ppm in herbivores, 1 ppm in carnivores, and 4 ppm in top carnivores. These degrees of biomagnification over this period of time after beginning of application are very reasonable for chlorinated hydrocarbons in the environment. Figure 8 shows the recovery of the system for a 15-year period following suspension of application. The inertia effect in the food chain causes an overshoot of concentration in the top carnivore population.

In order to model possible chronic toxicity effects, a dose/response curve was synthesized as was suggested in Equation (4). Figure 9 illustrates this function. It provides a feedback from the contaminant concentration to the population dynamics, each of which is represented by its own set of differential equations. The manner in which the function was applied provided an exponential transition from the normal mortality rate to some elevated rate characterizing the full force of the lethal effect. This choice is analogous to chemical rate processes where a Maxwell-Boltzmann molecular energy distribution of energies interact with a step function occurring at a discrete energy threshold.

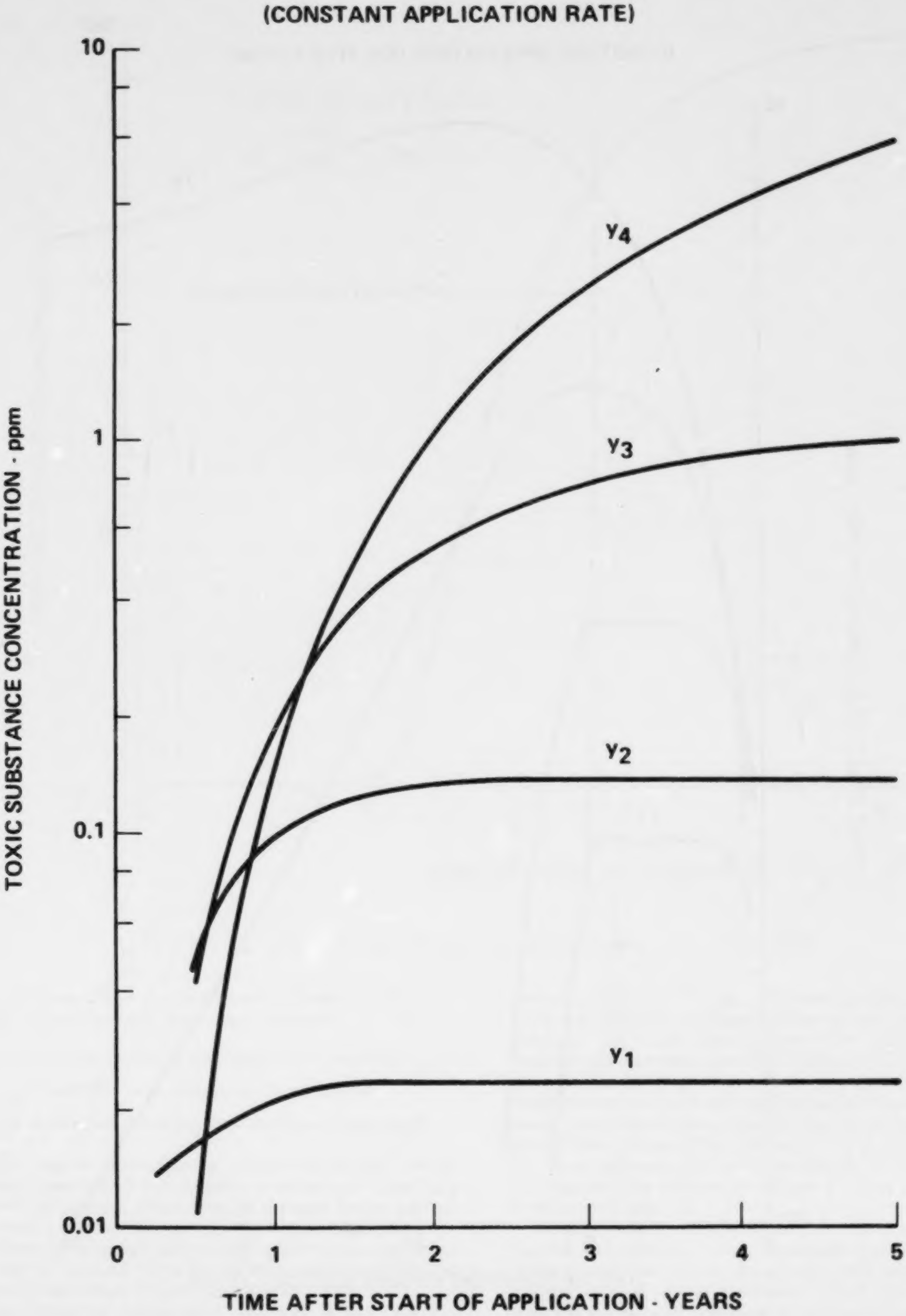


Figure 7. Dynamics of bioaccumulation modeled for the prototype ecosystem (Fig. 3) at constant application rate.

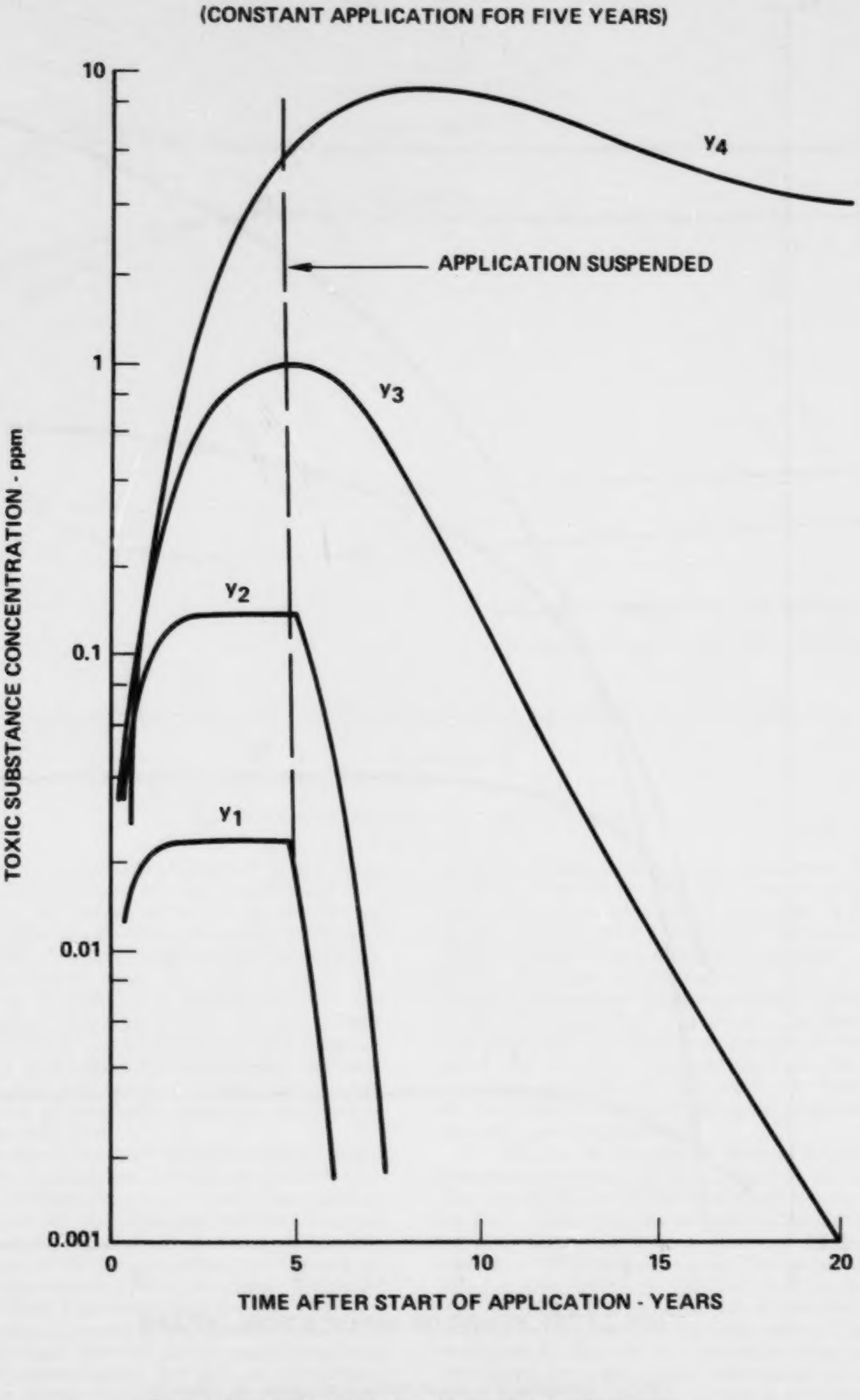


Figure 8. Bioaccumulation and decay for prototype aquatic ecosystem.

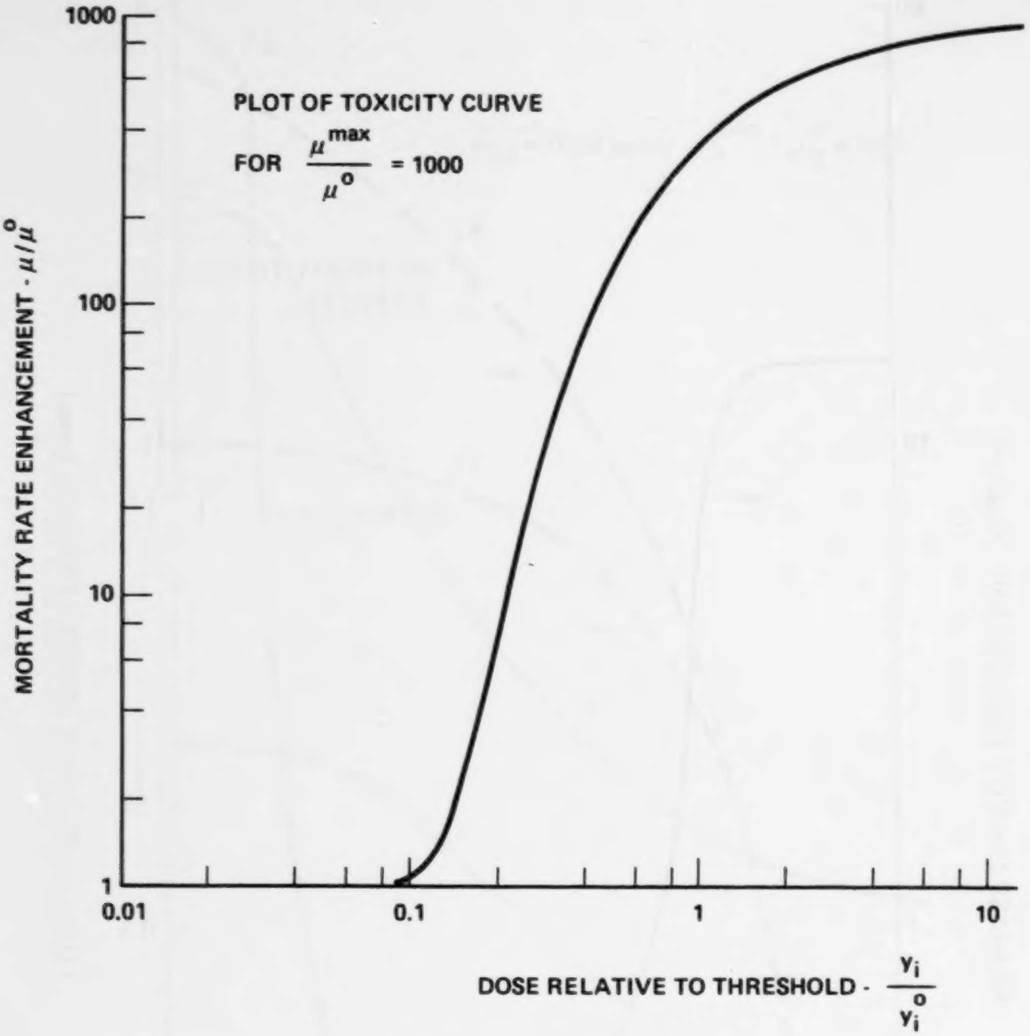


Figure 9. Assumed dose/response characteristic.

Three parameters are sufficient to describe this continuous sigmoid shaped dose/response curve:

a) baseline mortality rate coefficient (normal),

b) "threshold" concentration of contaminant,

c) saturation mortality rate coefficient (elevated).

Thus, this is equivalent to saying that among the individual members of a population compartment there is a Maxwell-Boltzmann distribution of dosages (dependent on individual variations of age, exposure, and metabolism). As this distribution moves to higher concentrations, ever larger fractions of the population exceed the critical value where mortality rate jumps to an elevated rate.

One numerical experiment was run to simulate the chronic lethal toxic effect by first imposing an input of contaminant for five years and then removing the source of toxic material. The source flux adjustment described above was employed and all of the rate coefficients were kept at normal levels except for the dose/response effect. This mortality rate behavior was assumed for the top carnivore compartment with a saturation mortality 10^3 times the normal baseline spontaneous mortality with a "threshold" of 1 ppm. At this point, the biomass of top carnivores dies back nearly two orders of magnitude during the second and third years. The contaminant concentration in the top carnivore compartment became self-limited at about 1 ppm because the lower biomass decreased the integrated ingestion rate and the mortality loss rate is enhanced. These effects are illustrated in Figure 10.

Another numerical experiment was conducted by imposing the dose/response effect described above on the herbivore population. Again the μ^{max}/μ^{o} ratio was set at 10^3, but the level at which significant effect sets in, y_i^{o} was assigned the value of 0.05 ppm. As expected, the damage to the ecosystem shows a much faster response than that in the case of the top carnivore mortality enhancement. Figure 11 shows the successive decreases in biomass energy equivalent among the populations above the producer level.

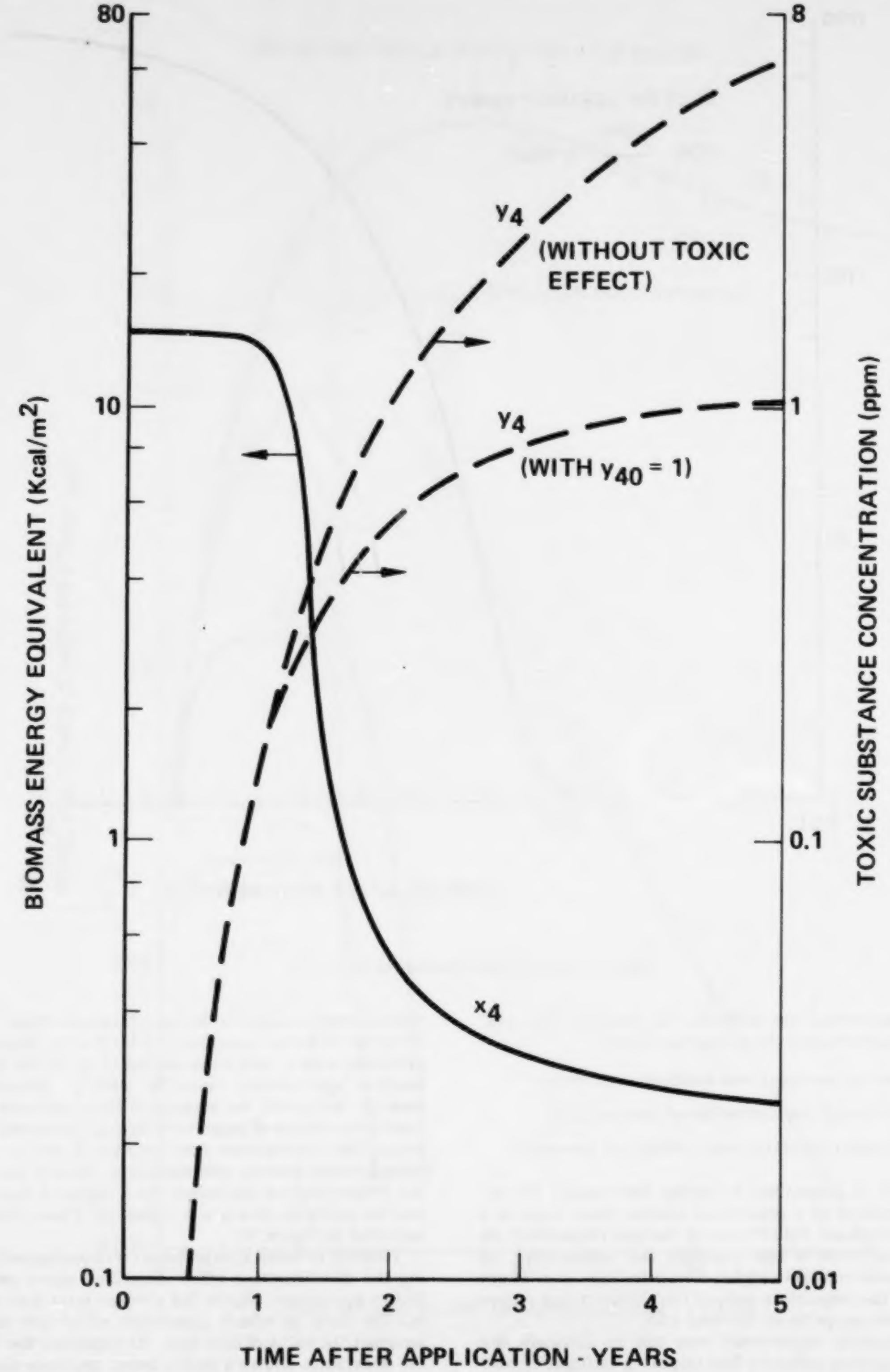


Figure 10. Toxic effect on top carnivores in prototype aquatic ecosystem (Fig. 3).

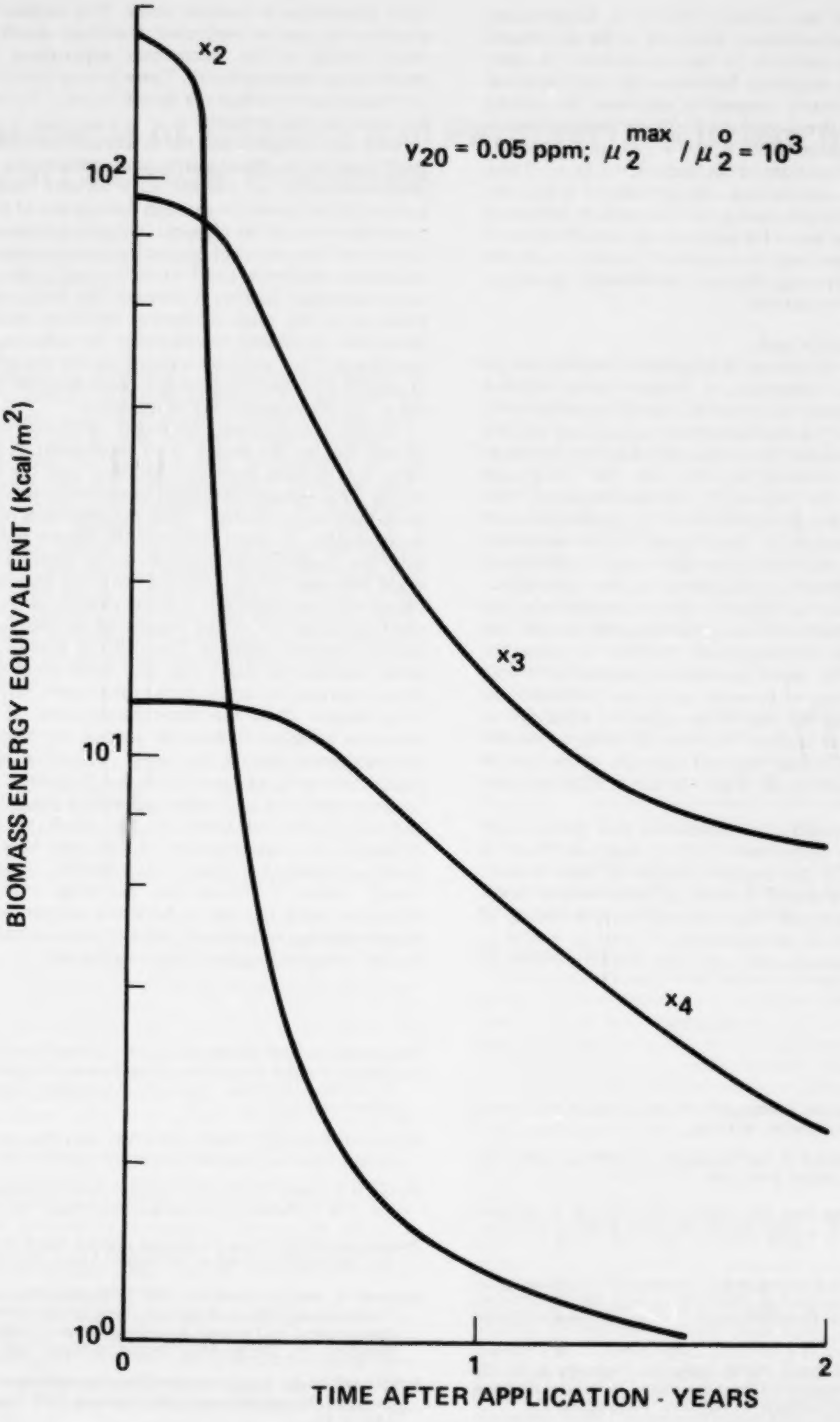


Figure 11. Toxic effect on herbivores in prototype aquatic ecosystem.

Both the acute and chronic response simulations demonstrate the applications of modeling to the movement and effects of toxic materials in the environment. In some cases of ecosystem modeling literature, the mathematical difficulty of numerically integrating nonlinear interacting systems has been overemphasized. These demonstration simulations had nonlinearities of the x^2, xy, and exponential type in a set of equations of the forms $\dot{x}_i = f_i\,(x_j, y_k, t)$ and $\dot{y}_i = g_i(x_j, y_j, t)$. The simulations were performed in seconds on a CDC 6400 central processing unit for years of simulated time. Multiplicity of boxes for geographical specification of the system is the next step of complexity. Indeed, with the present level of computing efficiency established, these elements of realism are feasible.

CONCLUDING REMARKS

Considering the legislative mandates controlling the introduction of new substances, a fruitful future role of mathematical modeling is a coordination of this effort with the biological modeling and laboratory microcosms studies to predict environmental consequences. Also the environmental chemistry studies provide data for computing transport through the physiochemical environment. The formal mathematical representations in the models generate enormous requirements for data inputs. Actual measurements are the best base for testing the model. Dependence upon these measurements by modelers requires certain protocols to be observed in obtaining environmental data. For example, many of the environmental chemistry parameters now contemplated or employed involve equilibrium phenomena such as vapor pressure or solubility. These coefficients are ratios of forward to reverse rates and are useful for calculating the rate in one direction when that in the other direction is known. Therefore, in order to provide dynamic models with their required data, one of the rates is desirable in addition to the value of the equilibrium constant.

The need for dynamic measurements also carries over into the laboratory microcosm work. A single-end state is usually measured in the reported results of these experiments. It would be useful if states at intermediate times could also be determined. Since non-destructive testing of the system is difficult or impossible, it may be useful to employ parallel systems and to analyze the distribution of toxic substances at various times. This implies that these experiments can be replicated; therefore, another requirement arising in the microcosm experiment is that of establishing reproducibility. There is very little to be gained in attempting to calibrate a model beyond the certainty of the experimental results.

Both the biological and the mathematical models will be used to establish quantitative connections with real world observations in the natural environment. One hope for mathematical models is through description of the material flows occurring in the physical/chemical environment, they can extend the results of laboratory measurements (both for elementary processes and for microcosms) to the problem of toxic substance pathways through the environment. Key receptors of the toxic substance, therefore, must be identified and monitored continuously for substances already introduced. This provides a data base for checking validity of models and thereby gaining a more detailed understanding of the phenomenology in question.

Ideally, the procedure for model validation follows very closely that for the design of an experiment. A statistically valid data sample must be identified, and the methods of taking the measurements must be audited carefully to determine their uncertainties. Then the operation of the model must employ as many coefficients known independently from the fundamental experiments as possible. Otherwise many free parameters exist and the study becomes a curve-fitting exercise masked as a model validation. To avoid this, coefficients in the model should be determined independently whenever possible. The utility of models for the purposes enumerated above will only come about when confidence is gained by actual tests of their performance against observations. Thus the mere construction of the model serves as a logical framework guiding the choice of what measurements should be taken. Objective performance parameters such as correlation coefficients and residuals between observed and computed values should be derived and should be demanded by the users of any model. Moreover, the uncertainties in the data base should be clearly displayed. These requirements underscore the strong needs for integrating modeling with field and laboratory work in order to have any substantial impact on standardization of protocols for evaluating substances prior to their introduction into the environment.

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Assessments of Volatility and Partition Coefficient from Physical Chemical Properties

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ABSTRACT

The knowledge of a chemical's volatility is essential in assessing the amount of chemical distributed between air and other phases of the environment. Since the amount of chemical in the atmosphere affords a direct exposure to human and other living organisms, assessment of the chemical's volatility and bioaccumulation potential from its physical and chemical properties would be of considerable interest in a terrestrial system. In this work: 1) a Langmuir-type equation is developed to equate the experimental evaporation rate of a chemical into air which can be successfully extended to a multicomponent system by the application of Raoult's law; and 2) a relationship is observed between octanol/water partition coefficients and vapor pressures of chemicals which contain identical hydrophilic groups.

INTRODUCTION

As a chemical is introducted into one part of the environment, it will be transported and distributed in air, water, soil, and the biological system. The extent to which it will become an environmental contaminant in these phases of the environment depends on the properties of the chemical and the interaction of these properties with environmental factors.

The concern over the human and environmental exposure to a variety of agricultural and industrial chemicals used has led to extensive studies in the transport, persistence, and accumulation potential of these chemicals. Since the vaporization of a chemical leads to its subsequent aerial transport, a knowledge of the chemical's volatilization is highly important for assessing the amount of chemical in the atmosphere as well as the extent of possible human exposure during manufacture and use.

Although there have been a few reports in volatility studies (Acree et al., 1963; Hartley, 1969; Mackay and Wolkoff, 1973; Dilling et al., 1975; Haque and Kohnert, 1976), there is presently insufficient information to give a quantitative assessment of the volatilization rate. It is, therefore, of interest to investigate the properties of a chemical and other physical factors governing volatility.

The work of Hartley (1969) reveals that, under a given atmospheric condition, the relative evaporation rate of a chemical can be satisfactorily related to the product of the vapor pressure and the square root of the molecular weight. However, a computation of the absolute rate would involve determination of less accessible quantities such as the diffusion coefficient and, particularly, the thickness of the stagnant air layer through which vapor diffusion takes place.

In the present work, an analysis of the experimental rates of evaporation for the selected organic chemicals indicates that a Langmuir-type equation can be used to account for the observed rates. Most chemicals selected for this study have been considered as either known or suspected hazards to human health and/or the environment (Stephenson, 1975). Since these chemicals are very volatile (vapor pressures at 24°C from about 1 to 200 mm Hg), analysis by the gravimetric method is most suitable.

As many industrial products are often a mixture of several components, it is desirable to study the evaporation rate from a multicomponent system to determine how each component contributes to the observed vapor loss. Several artificial binary and tertiary organic systems are designed for this study.

An assessment of a chemical's volatility and its bioaccumulation potential would seem to be of particular interest in a terrestrial system. The octanol/water partition coefficient has been used to estimate the relative strength of hydrophobic bonding and bioaccumulation (Helmer et al., 1968; Lu and Metcalf, 1975; Metcalf et al., 1973; Neely et al., 1974). Based on the available literature data, the present work shows that the partition coefficient can be related reasonably well to the vapor pressure of chemicals which contain identical hydrophilic groups.

EXPERIMENTAL

Evaporation rates from single-component and multicomponent organic liquids into air have been measured gravimetrically at room temperature (23.7 ± 0.5°C). Stainless-steel planchets (4.60 cm^2) with a wall height of 6 mm were used as the sample-containing vessels. About 1 gram sample was added to the planchet with a resulting sample level of about half the wall height. The planchet was then placed on the balance pan of Mettler H54 which, with its doors fully open, was located at the corner of the room where air flow is minimal. A stopwatch was used to record the time for a certain weight change.

For the study of evaporation rate from a mixed organic liquid, a solution with predetermined mole fractions was prepared. A sample of the solution was then transferred quickly with a pipette to the planchet on the balance pan. Normally, the sample was exposed to air for less than a minute before the balance was fully set for recording weight loss. In some cases, relatively more weight loss from the more volatile components of a multicomponent system before weighing proceeded would correspond to about 2-3% change in mole fractions in one minute. We made no correction for this variation but accepted the molar fractions of the prepared solution.

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The vapor pressure data of the selected organic chemicals were obtained from the Handbook of Chemistry and Physics (49th edition). All test chemicals were used as received from commercial sources, which had purities ranging $>96\%$.

The partition coefficient values in Table 3 were taken from the compilation of Leo et al. (1971) and vapor pressures from the source mentioned above unless otherwise specified. Some reported vapor pressures at 25°C were obtained by extrapolation across melting points along with an approximation that ΔH (fusion) $= 0.25\ \Delta H$ (evaporation) when solid—vapor equilibrium vapor pressures were not available. These data were shown in Table 3 with parentheses. Although some error is introduced by this procedure, the extent of which is believed to be far less than an order of magnitude.

Table 1—Volatilities of Organic Chemicals at 23.7 ± 0.5°C

Compound	$Q_{exp} \times 10^5$ (g/cm^2 - sec)	$F = P(M/2\pi RT)^{1/2}$ (g/cm^2 - sec)	$(\beta = Q_{exp}/F) \times 10^5$
Chloroform	13.2	6.84	1.93
Carbon tetrachloride	9.23	4.41	2.09
1.1.1-Trichloroethane	9.04	4.68	1.93
1.2-Dichloroethane	5.20	2.66	1.96
Trichloroethene	5.07	2.79	1.77
Benzene	4.82	2.72	1.77
1.2-Dichloropropane	3.15	1.78	1.77
p-Dioxane	1.80	1.14	1.58
Tetrachloroethene	1.36	0.740	1.84
1.2-Dibromoethane	1.24	0.625	1.98
m-Xylene	0.501	0.280	1.79
Water (into R.H. = 30)	0.504	0.221[a]	2.28[a]
1.1.2.2-Tetrachloroethane	0.448	0.263	1.70
1.2-Dichlorobenzene	0.118	0.053	2.21

[a] Corrected for 30% relative humidity of ambient air.

Table 2—Volatilities of Mixed Organic Liquids at 23.7 ± 0.5°C

	Q_{exp} (g/cm^2- hr)	Q_1 (g/cm^2 - hr)	Q_2 (g/cm^2 - hr)	Q_{exp}/Q_1	Q_{exp}/Q_2
Binary System					
Chloroform/1.2-DBE (0.517/0.483)[a]	0.324	0.267	0.257	1.21	1.26
1.2-DCE/1.2-DBE (0.523/0.477)	0.151	0.119	0.116	1.27	1.30
1.2-DCE/1.2-DCP (0.552/0.448)	0.159	0.154	0.155	1.03	1.03
1.2-DCE/1.1.2.2-TCEA (0.573/0.427)	0.113	0.114	0.112	0.991	1.01
TCEE/1.1.2.2-TCEA (0.508/0.492)	0.0432	0.0328	0.0346	1.32	1.25
Tertiary System					
Chloroform/Benzene/1.2-DBE (0.351/0.320/0.329)	0.312	0.237	0.238	1.32	1.31
1.1.1-TCE/1.2-DCP/TCEE (0.333/0.343/0.324)	0.154	0.163	0.165	0.945	0.933

[a] Numbers in parentheses refer to mole fractions.

DBE = dibromoethane; DCE = dichloroethane; DCP = dichloropropane; TCE = trichloroethane
TCEA = tetrachloroethane; TCEE = tetrachloroethene

$Q_1 = \Sigma X_i Q_i^0$; $Q_2 = \overline{\beta} \Sigma X_i F_i^0$; Q_{exp} = experimental rate.

Table 3—Vapor Pressures and Partition Coefficients

	log K	log P (25°)
Aromatic Hydrocarbons		
Benzene	2.13	1.98
Toluene	2.69	1.42
3-Xylene	3.20	1.11
4-Xylene	3.15	1.08
Naphthalene	3.37	−1.10[a]
Biphenyl	4.04	−2.04[b]
Phenanthrene	4.46	−3.77[b]
Anthracene	4.45	−5.15[b]
Organohalogens		
Chloroform	1.97	2.29
Carbontetrachloride	2.64	2.04
Ethyl iodide	2.00	2.11
1-Bromopropane	2.10	2.13
Tetrachloroethene	2.60[i]	1.27
Fluorobenzene	2.26	1.88
Chlorobezene	2.84	1.04
Iodobenzene	3.25	0.079
Bromobenzene	2.99	0.623
1,2-Dichlorobenzene	3.38	0.114
1,3-Dichlorobenzene	3.38	0.342
1,4-Dichlorobenzene	3.39	0.00[g]
4,4'-PCB	5.58[k]	−4.71[c]
p,p'-DDE	5.69[e]	−5.19[d] (30°)
p,p'-DDT	6.19[e]	−6.48[f]
Aliphatic Alcohols		
Methanol	−0.82	2.09
Ethanol	−0.32	1.75
Propanol	0.34	1.28
Butanol	0.88	0.845
Pentanol	1.40	0.380
Hexanol	2.03	0
Octanol	3.15	−1.00
Dodecanol	5.13	−2.05
Aliphatic Acids		
Acetic acid	−0.17	1.18
Propionic acid	0.33	0.625
cis-Crotonic acid	0.72	−0.292
Butyric acid	0.79	−0.0362
Hexanoic acid	1.88	−1.82
Decanoic acid	4.09	(−3.40)[j]
Lauric acid	4.20	(−3.26)
Palmitic acid	5.31	(−4.35)
Chlorinated Phenols		
2-Chlorophenol	2.15	0.301
3-Chlorophenol	2.50	(−0.658)
4-Chlorophenol	2.39	(−0.886)
2,4,5-Trichlorophenol	3.72	(−1.64)
2,4,6-Trichlorophenol	3.69	(−1.80)
Pentachlorophenol	5.01	−3.80[h]

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[j] Figures in parentheses are calculated according to the method described in text.

[k] Measured value from this laboratory.

RESULTS AND DISCUSSION

Evaporation Rate

Rates of evaporation from single-component systems for thirteen organic chemicals and water are shown in Table 1. A related plot of weight loss versus time is presented in Figure 12. Analysis of the data indicates that a basic Langmuir-type equation can be related to the observed rate (Q) as follows:

$$Q = \beta P(M/2\pi RT)^{1/2} = \beta F \qquad (1)$$

In equation 1, β is an evaporation constant for a chemical under a given set of atmospheric conditions, P the effective vapor pressure (which is equal to saturated vapor pressure Po in a one-component system), M the molecular weight, and R and T the gas constant and ambient temperature, respectively. The determined β values for various compounds as indicated in Table 3 show only a small variation

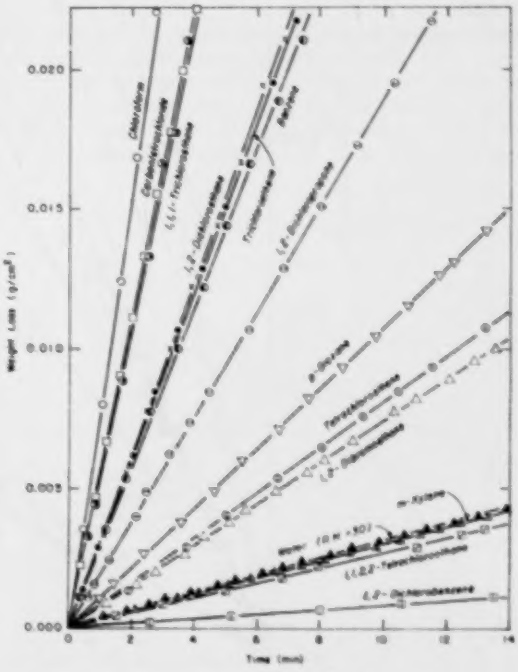

Figure 12. Evaporation loss of organic chemicals from one-component systems at 23.7 ± 0.5°C.

and have an average $\overline{\beta} = 1.90 \times 10^{-5}$ with a standard deviation of 10%. The presence of small amount of impurities in the test samples may have caused some degree of variation in β values. The present $\overline{\beta}$ value is in close range with the evaporation data of Hartley (1969) which, after similar calculations by the authors, would correspond to a $\overline{\beta} = 1.41 \times 10^{-5}$ for water, 2,2,4-trimethylpentane, m-xylene, and anisole evaporating under a still air at 20°C of ambient temperature.

Equation 1 can be applied to describe the volatility rate of a multicomponent system using Raoult's law. In this case, the effective vapor pressure of component i is equal to the partial pressure of this component assuming that the concentration in the liquid phase is uniform.

$$P_i = P_i X_i \gamma_i \tag{2}$$

The total evaporation rate is the sum of the rates of all components. Thus,

$$Q = \Sigma Q_i = \Sigma X_i \gamma_i Q_i^o \tag{3}$$

where X_i and γ_i are the mole fraction and activity coefficient of component i in the system, and Q_i^o the single-component rate of component i. When equation 1 is substituted, equation 3 can be expressed as:

$$Q = \beta \Sigma X_i \gamma_i F_i^o \tag{4}$$

where $\overline{\beta}$ is used instead of β_i because of the relative invariability of this quantity in the present case. F_i^o is calculated using P_i^o. To simplify the rate calculation, we have neglected γ_i in equations 3 and 4. However, a comparison of the measured rate with that predicted from either of these two equations would give an indication on the ideality of a given solution system. Experimental and estimated rates from equations 3 and 4 are shown in Table 2 for five binary and two tertiary organic systems. A plot of the weight loss versus time for these multicomponent systems is shown in Figure 13.

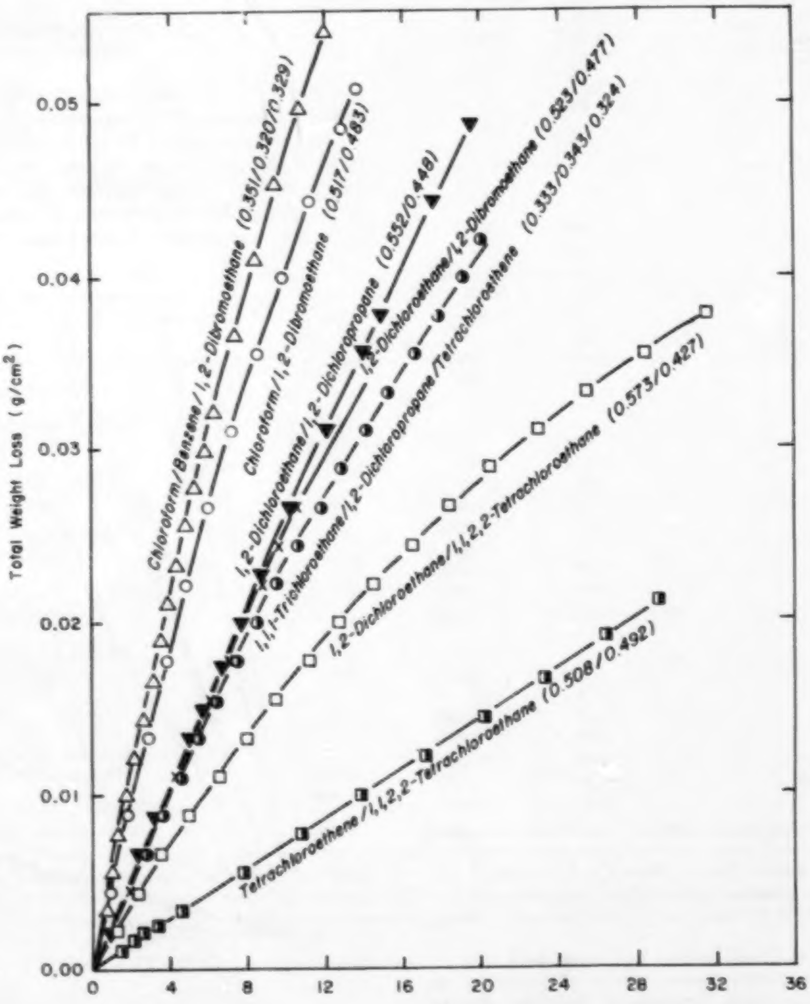

Figure 13. Evaporation loss of organic chemicals from multicomponent systems at 23.7 ± 0.5°C.

The rates reported in Table 2 were taken when the evaporation time was approaching zero since our predicted rates were based on initial mole fractions of the system. The non-linear behavior of the evaporation curve for a multicomponent system is a result of instantaneous change in mole fraction of the system during the course of evaporation. For a system containing a volatile component and a relatively less volatile one such as 1,2-dichloroethane/1,1,2,2-tetrachloroethane, a more pronounced effect is observed due to a more rapid change in mole fraction. On the other hand, a combination of two components of about equal volatility such as 1,2-dichloroethane/1,2-dichloropropane showed a relatively constant rate. In general, good agreement has been observed between measured and predicted rates for the seven multicomponent systems as can be seen from the ratios of these two rates. This suggests that equation 3 or 4 effectively accounts for the evaporation rate from a multicomponent organic system. The results also indicate that the activity coefficients for components in these systems are fairly close to unity.

In principle, one can predict the instantaneous evaporation rate of the system using the data of single-component rates (Q_i^0) and a knowledge of the instantaneous mole fractions of each component during evaporation. With known initial weight and mole fraction for each component, a calculation of instantaneous X_i can be made by adjusting for the weight loss during each small time interval (Δt). From this, the accumulated total weight loss can be plotted against time. The calculated evaporation curves, based on this method (neglecting γ_i and using $\Delta t = 1$ min) for 1,2-dichloroethane/1,1,2,2-tetrachloroethane and 1,1,1-trichloroethane/1,2-dichloropropane/tetrachloroethene (not shown here), showed very close agreement with the experimental results. The results suggested that the mixing time required to transport a material from bulk organic liquid to the evaporating surface appeared to be very rapid. This was presumably due to a high degree of solution ideality and a relatively shallow liquid depth ($\leq$ 4mm), so that no significant concentration gradient was established during evaporation.

Our work which is presently in progress indicates that the evaporation constant β is almost completely independent of temperature and air humidity. The variation of β under atmospheric conditions seems to be mainly dependent on the velocity and type of air flow across the evaporating surface and the heat supply to the system. The work of Hartley (1969) indicated many-fold increase in the evaporation rates of relatively low volatile compounds when fanning was applied to the system; the increased rates seemed fairly constant for these compounds (e.g., m-xylene, anisole, and n-octanol) which required less heat for evaporation. More extensive studies would be needed to establish a quantitative dependence of β value on the air flow rate.

Partition Coefficient and Vapor Pressure

A linear free-energy relation between the octanol/water partition coefficient and aqueous solubility for many classes of monofunctional organic liquids has been reported (Hansch et al., 1968). In this report, most partition coefficients were assessed from the π constants of the substituents in relation to some parent compounds since the experimental values were not measured. This relationship was extended (Chiou et al., 1977) to cover compounds of varying structural diversity, including compounds such as organophosphates and PCBs, using the experimental partition coefficients.

These findings suggest that the extent of aqueous solubility of an organic chemical provides a good measure of its partition coefficient, and thus its bioaccumulation potential. It also implies that the partitioning of an organic chemical between octanol and water could be considered equivalent to that between the chemical itself and water (i.e., solubilization).

As an extension of the reasoning given above, one might consider the partitioning of a chemical between itself and its vapor at equilibrium (i.e., vapor pressure) to be equivalent in nature to that of solubilization, if an adjustment is made for the hydrophilic contribution of certain functional groups in the molecule. Based on this, a linear free-energy relationship between the octanol/water partition coefficient and vapor pressure would be expected. The condition can be largely met for chemicals within the same family which contain identical hydrophilic groups (e.g., -OH, -COOH, -NO$_2$, etc.), neglecting other molecular properties which may also be important.

Table 3 shows the partition coefficients (K) and vapor pressures (P) for five classes of organic chemicals—aromatic hydrocarbons, organohalogens, aliphatic alcohols, aliphatic acids, and chlorinated phenols. The classification of organohalogens assumes that the hydrophilic contribution due to unsaturated bonds is relatively small in comparison with other potent hydrophilic groups. All halogenated organic chemicals are thus grouped without regard to whether they belong to aliphatic or aromatic class.

As shown in Figure 14, a reasonably good fit is observed between log(K) and log(P) for the selected chemicals. A decrease in vapor pressure in each family is accompanied by a proportional increase in partition coefficient. This increase is more rapid for alcohols and acids, indicating a more rapid transition from hydrophilic to hydrophobic characteristics. No appreciable difference is noted between organohalogens and aromatic hydrocarbons. This supports the assumption made earlier that unsaturated bonds make relatively small contributions to hydrophilicity. It is of interest to recognize that at vapor pressure ≥ 10 mm Hg at 25°C the partition coefficients of these two families are

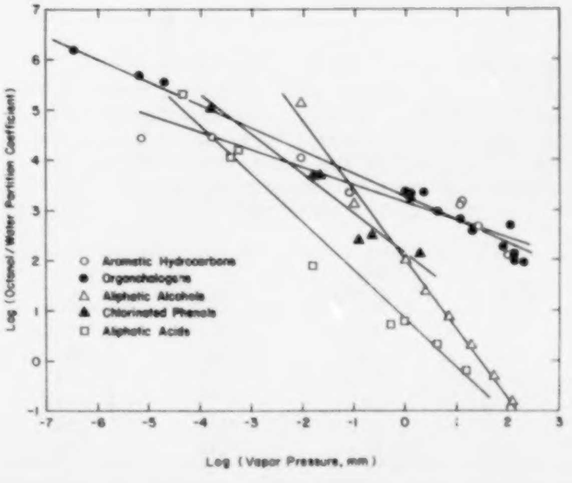

Figure 14. The relationship between octanol/water partition coefficients and vapor pressures of organic chemicals.

many orders of magnitude higher than those of other families. Numerous industrial products with estimated annual release rates from tens to hundreds of millions of pounds a year (e.g., chloroform, carbon tetrachloride, 1,2-dibromoethane, benzene, etc.) fall into this category (Stephenson, 1975). In consideration that such volatile compounds also possess relatively higher partition coefficients, the extent of human exposure to these chemicals is likely to be greater. A correlation relating partition coefficients to vapor pressures like the one shown in Figure 14 would be of value to assess a chemical's bioaccumulation potential relative to its volatility in a terrestrial system.

The relation of the partition coefficient to aqueous solubility and to vapor pressure indicates that, within a chemical family, these three physical properties are interrelated such that as a chemical's solubility decreases, there is a corresponding decrease in vapor pressure and an increase in partition coefficients, i.e., bioaccumulation potential.

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Partition Coefficient and Environmental Fate of Xenobiotic Compounds

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ABSTRACT

The lipid/water partition coefficient is a critical parameter in determining the uptake of xenobiotic compounds by living organisms in the aquatic and terrestrial environments and their biomagnification through food chains. This is discussed from both the environmental and theoretical viewpoints. The accurate measurement of lipid/water partition values presents many difficulties especially for highly lipid soluble compounds. These are illustrated and discussed.

INTRODUCTION

The ability of living organisms to concentrate and accumulate relatively high concentrations of lipid soluble organic compounds either directly from water or indirectly through consumption of contaminated food web organisms, is perhaps the most serious problem resulting from environmental pollution by organic micropollutants. This process, termed bioconcentration or ecological magnification is of major importance in determining the safety of the human food supply, e.g. consumption of tuna fish contaminated with methyl mercury from the ocean; coho salmon, lake trout, and chubs contaminated with DDT, dieldrin, and polychlorinated biphenyls (PCB) from Lake Michigan (Environmental Protection Agency, 1972); cow's milk contaminated with polybrominated biphenyls (PBB) (Anon., 1975); or chickens contaminated with dieldrin from feeding on soybean oil sludge (Anon., 1974). Human milk, containing an average of 3.5% lipids and originating at the top of the food web, is inevitably contaminated with all the stable, lipid soluble organic compounds entering the human dietary and these are readily passed along to the nursing offspring solely dependent upon this often suspect dietary source. Thus, recent surveys have suggested that the average U.S. breast fed infant ingests 2X the FAO-WHO Acceptable Daily Intake (A.D.I.) for DDT-DDE and 10X the A.D.I. for dieldrin (Curley and Kimbrough 1964).

Thus, the lipid/H_2O partition coefficient of organic pollutants is a critical environmental parameter in determining bioconcentration (Neely et al. 1974, Lu and Metcalf 1975, Chiou et al. 1977). An even simpler environmental parameter, water solubility shows a high inverse correlation with bioconcentration (Metcalf and Sanborn 1975, Chiou et al. 1977), and this clearly reflects the contrasting lipophilic and hydrophilic molecular properties.

These few examples of the problems resulting from widespread environmental contamination by organic compounds with high lipid/water partition values lend emphasis to Abelson's (1970) suggestion that companies producing fat-soluble, non-biodegradable organic chemicals should carefully consider what they may responsibly set loose on the environment. The Environmental Protection Agency has proposed evaluation of bioconcentration as a major criterion in establishing effluent standards for pollution, and determination of lipid/H_2O partition coefficient, one of the requirements for registration of new pesticides (Quarles 1973).

QUANTITATIVE ASPECTS OF BIOCONCENTRATION

Absorption Through Integument

This phenomenon is of critical importance to aquatic organisms exposed to trace amounts of stable xenobiotics with high lipid/H_2O partition coefficients. Fish in abyssal lakes and oceans where detoxication processes are minimal, continue to absorb and store such compounds as DDT, dieldrin, and PCB's over their entire life span. The following bioconcentration factors have been demonstrated for the mature lake trout *Salvelinus namaycush* in Lake Michigan. (EPA 1972):

	average - ppm		*bioconcentration*
	lake	trout	*factor*
DDT(T)	0.0000060	18.80	3.13×10^6
dieldrin	0.0000020	0.26	1.30×10^5
PCB's	0.0000082	28.0	3.41×10^6

In Lake Cayuga, Youngs et al. (1972) found that as lake trout increased in age the body residues of DDT-(T) increased from approximately 1.0 ppm at 1 year to 16-20 ppm at 11 years. The correlation between age and total body residue for a three year period, 1968 to 1970, was r = 0.93. Lake trout in Lake Michigan showed a similar correlation between size and total body residues (EPA 1972):

size - inches		*residue - ppm*	
		DDT-(T)	dieldrin
2.0	5.9	0.89	0.03
6.0	9.9	2.24	0.12
10.0	15.9	6.00	0.14
16.0	21.9	8.00	0.21
22.0	26.9	14.62	0.23
27.0	32.9	19.23	0.26

The correlations were excellent (DDT $r = 0.986$, dieldrin $r = 0.962$).

Since the concentrations of these xenobiotics in large bodies of water were essentially constant, it is clear that bioaccumulation in aquatic poikilothermic animals is proportional to the concentration in water x time of exposure or Ct. This is shown in Figure 15 where the storage of DDT by the fish *Tilapia mossambica* and *Lepomis cyanellus* fitted this Ct relationship very well (Reinbold et al. 1971).

This absorption is therefore a first-order process, i.e. the amount penetrating per unit time is a function of the amount at the absorbing surface. This absorption process has been expressed as a form of Fick's law (Buerger 1966):

$$\frac{dS}{dt} = -DA\frac{dC}{dX} \qquad (1)$$

where D is the diffusion coefficent, A is the cross-sectional area of surface, X is the thickness of surface (constant), C is the concentration of the diffusing substance, and S is the total amount of a diffusing substance present at the surface. Buerger (1966) has concluded that penetration will follow first-order kinetics in a biological integument composed of any number of phases structured in any possible arrangement.

Pharmacodynamics of Bioconcentration

The quantitative aspects of xenobiotic absorption and metabolism by organisms living in a large pool of constant contamination, e.g. fish in an abyssal lake or earthworms in a field treated with pesticide, represent an example of the compartmental analysis of the pharmacokinetics of drug absorption and metabolism (Goldstein et al. 1974, Kerr and Vass, 1973). Let us assume that the organism is represented by a single homogenous compartment of constant size V (organism mass or volume), and the xenobiotic is absorbed at a constant zero-order rate k_a, determined by the lipid/H_2O partition coefficient, from the surrounding environment contaminated at a constant level C_e. The concentration in the organism C_o can then be represented as:

$$\frac{dC_o}{dt} = \frac{C_e k_a}{V} - k_c C_o \qquad (2)$$

Here k_c is the rate constant for clearance of the xenobiotic by the processes of degradation, elimination and "growth dilution." This combination occurs at a rate proportional to the internal concentration in the organism C_o, and has first-order kinetics.

Integration of Eq. 2 gives the concentration in the organism at any time t as:

$$C_o = \frac{C_e k_a}{k_c V}(1-e^{-k_c t}) \qquad (3)$$

If the rate of absorption of the xenobiotic into a living organism is a constant and the rate of output is exponential, then the concentration in the organism (C_o) will increase until a steady state is reached where clearance is equal to absorption. Thus as t becomes infinite, $1-e^{-k_c t}$ becomes unity, and at the steady state:

$$C_o = \frac{C_e k_a}{k_c V} \qquad (4)$$

This is called the plateau principle (Goldstein et al. 1974).

However, in poikilothermic aquatic organisms such as fish, where k_c is determined by enzymatic processes influenced by environmental temperatures, the rate of clearance (k_c) becomes very small in comparison with the rate of absorption (k_a) that is largely independent of temperature, except as a function of respiratory rate. Therefore, the length of time required to reach a plateau or constant residue level will often exceed the longevity of the organism. This phenomena can become of critical importance with the very stable organochlorine xenobiotics such as DDT and PCB's where lipid/water partition values are very

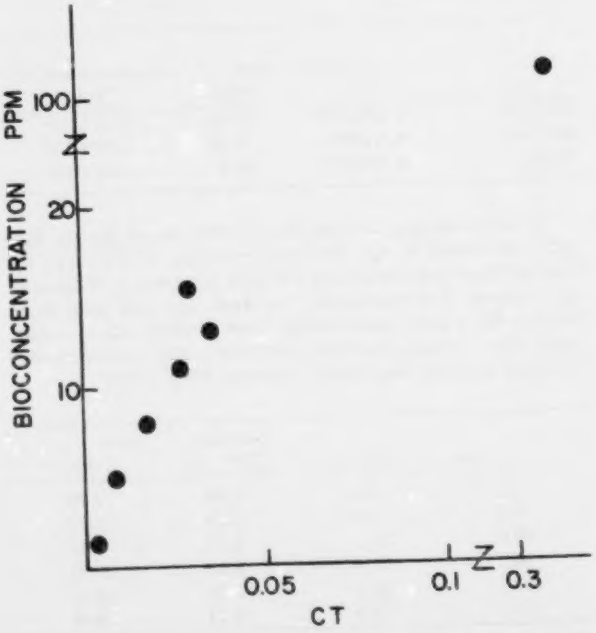

Figure 15. Relationship of bioconcentration of ^{14}C DDT in the fish *Tilapia* to concentration in water (ppb) × time (days) or CT. (After Reinbold et al. 1971).

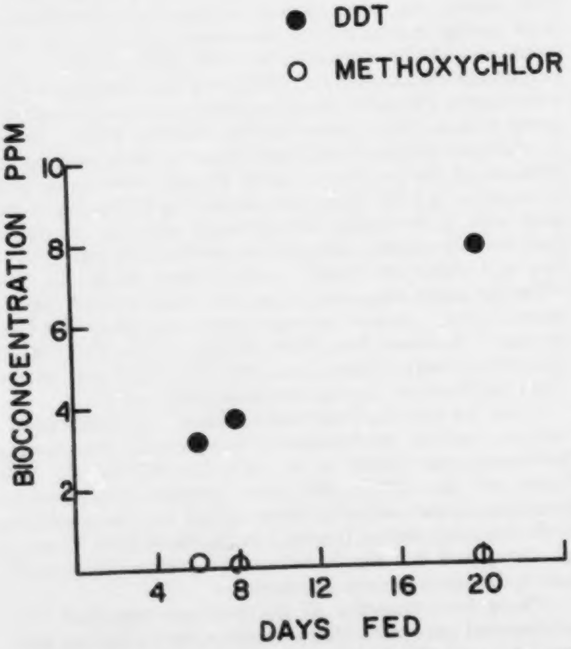

Figure 16. Uptake of ^{14}C DDT and ^{3}H methoxychlor by guppies fed daphnia containing 25-26 ppm DDT or 21-22 ppm methoxychlor (After Reinbold et al. 1971).

high and elimination is very slow. The lake trout in Cayuga Lake and in Lake Michigan where bioconcentration is a linear function of age, provides a convincing example. (Youngs et al. 1972, EPA 1972).

These equations are oversimplified in the assumption of constant parameters that are seldom found in most ecological systems. However, they are useful to describe the rates of bioaccumulation of xenobiotics both in artifically controlled systems and in large abyssal lakes or oceans where constant conditions are more likely to prevail. They are clearly useful for the comparison of bioconcentration of various xenobiotics in different organisms and for evaluating additional environmental factors that can make them less applicable in a variety of specific situations.

Bioconcentration from Food

The rate of xenobiotic accumulation by ingestion of contaminated food or prey can be treated as a modification of Eq. (1) in which the factor for accumulation of the xenobiotic is the daily intake or concentration in prey (C_p) X weight of prey (W_p) X a suitable factor (c) to measure the efficiency of extraction from the food:

$$\frac{dC_o}{dt} = \frac{c(C_pW_p)}{V} - k_cC_o \qquad (5)$$

As shown in Figure 16, the guppy *Lebistes reticulatus* fed *Daphnia magna* contaminated with 25-26 ppm DDT, bioconcentrated the DDT at a nearly linear rate. However, when the guppy was fed *D. magna* contaminated with 21-22 ppm methoxychlor, which is readily cleared; bioconcentration was scarcely perceptible (Reinbold et al. 1971).

LIPID/WATER PARTITION COEFFICIENTS

The partition of chemicals between lipids and water determines the rate of entry of lipid soluble substances directly through the integument of animals immersed in substrates contamined with xenobiotics (see Eq. 2), from water to blood through gills, and from blood to tissues, or in the case of ingestion in food directly from intestine into blood.

To make quantitative comparisons of lipid/water partition ratios, early workers used chloroform, ether, or hexane as the lipid mimic. Fujita et al. (1964) proposed octanol as a more realistic lipid mimic and used the octanol/water partition coefficient as a constant to express the distribution of drugs in living systems. The log octanol/H_2O partition was defined as Π, a linear free energy parameter which can be approximated by

$$\Pi x = \Sigma \text{ substituent constants} + \Pi_H$$

where H is the value for benzene or a benzene derivative and X is a substituted benzene. Tables were compiled for a wide range of substituent groups attached to aromatic systems (Fujita et al. 1964. Hansch et al. 1973) and it was proposed that the Π value for any aromatic compound could be calculated from the additive nature of these substituents. Over a moderate range of Π values, the calculated octanol/H_2O partition values are in good approximation to those measured directly using radiolabeled compounds, as shown in Table 4. However, as partition coefficients increase, there is a greater and greater discrepancy between calculated and measured values. This is graphically shown in Table 5, for a series of polychlorinated biphenyls. Obviously, the calculated values for the more highly chlorinated biphenyls are so enormously high as to be unreasonable. This is indicated clearly in Figure 17 where the logarithm of ecological magnification (E.M.) as determined in the fish *Gambusia affinis* is plotted against the logarithm of the octanol/water partition coefficient. The values for partition coefficients as determined from radiolabeled compounds fit the ecological magnification data much more plausibly then the calculated values.

Determination of Partition Coefficients

The practical measurement of lipid/water partition coefficients presents substantial difficulties. The accuracy of measurement obviously decreases with the disparity in the amounts of solute in two phases, i.e. at very low and very high partition values. The latter are clearly those of greatest concern in environmental pollution. The choice of octanol as the lipid phase also complicates the determination as octanol and water are appreciably miscible and octanol-water emulsions are difficult to separate clearly.

Table 4—Comparison of Calculated and Measured Octanol/Water Partition Coefficients

	octanol/water partition	
	measured[1]	calculated[2]
aniline	7	8
benzidine	28	43
nitrobenzene	62	71
benzoic acid	108	71
anisole	119	129
chlorobenzene	150	692
trifluralin	1350	4260
pentachlorophenol	6405	707,946
hexachlorobenzene	13,560	2,454,709

[1]Lu and Metcalf (1975) and unpublished data, by radiolabel.
[2]Fujita et al. (1964).

Table 5—Comparison of Calculated and Measured Octanol/H_2O Partition Coefficients for Polychlorinated Biphenyls

	octanol/water partition determined		
	calculated	Metcalf et al. (1975)[1]	Chiou et al. (1977)[2]
4,4′-dichlorobiphenyl	323,594	7122	380,189
2,5,2′-trichlorobiphenyl	1,659,587	7803	—
2,5,2′5′-tetrachlorobiphenyl	8,511,380	8126	—
2,4,5,2′,5′-pentachlorobiphenyl	43,651,583	16,057	1,288,250
2,4,5,2′,4′,5′-hexachlorobiphenyl	223,872,115	29,956	5,248,075
octachlorobiphenyl	5,888,436,561	—	—
decachlorobiphenyl	154,888,166,180	—	—

[1]Values from radiolabeled compounds, Metcalf et al. (1975) and unpublished.
[2]Values from glc.

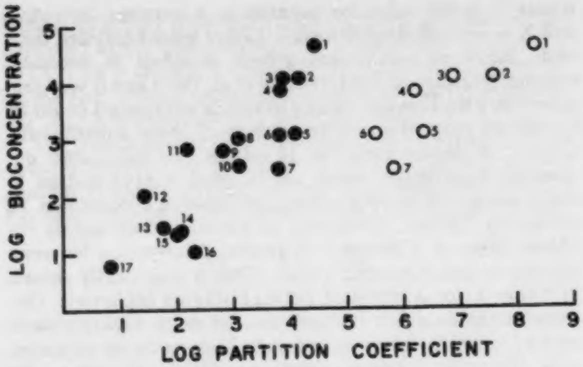

Figure 17. Relationship of log bioconcentration in the fish *Gambusia*, as determined by laboratory model ecosystem studies, to log octanol/water partition coefficient. Open circles calculated values, solid circles values determined by radiotracers. Compounds: (1) 2,4,5,2',4',5'-hexachlorobiphenyl, (2) 2,4,5,2',5'-pentachlorobiphenyl, (3) 2,5,2',5'-tetrachlorobiphenyl (4) 2,5,-2'-trichlorobiphenyl, (5) hexachlorobenzene, (6) 4,4'-dichlorobiphenyl, (7) pentachlorophenol, (8) trifluralin, (9) lindane, (10) parathion, (11) chlorobenzene, (12) benzidine, (13) nitrobenzene, (14) anisole, (15) benzoic adid, (16) atrazine, and (17) aniline. Data from Lu and Metcalf (1975), Metcalf et al. (1975), Metcalf and Sanborn (1975) and unpublished.

Traces of octanol containing large proportions of lipid partitioning substances can substantially distort the values obtained. Chiou et al. (1977) have emphasized the employment of redistilled octanol and high speed centrifugation to minimize this difficulty.

It is important to measure the amount of solute in both lipid and water phases, especially as the partition coefficient increases. Fujita et al. (1964) used the method of differences and determined only the amount in the water phase. This is satisfactory where the partition coefficient is low. This, however, gives meaningless results where the amount in octanol is 1000-fold or more greater than that in water.

The use of radiolabeled molecules provides the most convenient way to determine partition values. However, a very high radiopurity is required as traces of more water soluble starting impurities can subtantially increase the dividend and thus greatly decrease the quotient. Thus the use of radiolabeled products tends to give low values for very highly lipid soluble substances.

Gas liquid chromatography can give specific determination of the parent compound so that very high purity is not as critical as with radiolabeled compounds. However, for substances with very high partition values there are major difficulties in extracting the substance from trace amounts in the water phase into hexane for glc determination. Thus glc techniques tend to give high values for very highly lipid soluble substances.

Fortunately, for use in prediction of food chain transfer and biomagnification, knowledge of the exact magnitude of the lipid/water partition coefficient is not essential. Based on a great deal of observation in both the natural environment and in the laboratory, chemicals with lipid/water partition values greater than 1000-fold may be expected to cause some sort of environmental problems.

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Environmental Exposure Assessment Using Laboratory Measurements of Environmental Processes

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ABSTRACT

The objective of this study is to develop procedures for environmental exposure assessment of selected organic compounds that might be found as low level pollutants in freshwater aquatic systems. The compounds studied are p-cresol, quinoline, benzo[f]quinoline, benzanthracene, benzo[a]pyrene, carbazole, benzo[b]carbazole, dibenzo[c,g]carbazole, benzo[b]thiophene, dibenzothiophene, mirex and methyl parathion. Laboratory measurements of rates and equilibria were carried out for volatilization, sorption, hydrolysis, photolysis, oxidation and biodegradation. Screening studies provided a preliminary assessment of the relative importance of the competing processses. More detailed studies of the dominant processes were carried out, including product studies for chemical and microbial transformations. These data were used with a nine-compartment computer model to predict the transport, distribution and steady-state concentrations of these compounds in four representative aquatic environments. Results for seven compounds are reported here in terms of their distribution in solution, on solids and their half-lives in solution towards several competing processes in a eutrophic lake.

INTRODUCTION

Environmental exposure assessment uses the results of laboratory measurements and a computer model to predict environmental exposure to selected organic compounds in several kinds of freshwater aquatic environments.

This study was designed to achieve three objectives:

- Develop procedures for a general environmental exposure analysis of a chemical based on laboratory measurements of the rates of physical, chemical, and microbiological transformations of the chemical, which are believed to be important in the aqueous phase of natural freshwater ecosystems.

- Develop a computer integration procedure for extrapolating data from laboratory conditions to a variety of natural water conditions.

- Demonstrate these procedures using a series of selected organic chemicals.

Laboratory experiments were performed on homogeneous water solutions of selected chemicals below their solubility limits to measure the partition coefficients for sorption on sediments or biomass and the rates of volatilization, oxidation, hydrolysis, photolysis, and microbiological transformations of aquatic freshwater systems. The results of the laboratory studies were integrated with a simple nine compartment computer model to predict the fate of the chemicals in ponds, streams, and lakes.

A potential shortcoming of this approach to environmental assessment is that it may not take into account other important transformation or transport processes that occur in natural aquatic systems. To minimize this possibility, we have tried to validate idealized laboratory experiments conducted in pure water or with purified clays by also using natural sediments and waters for comparative measurements.

Three potentially important pathways were deliberately omitted from our model: chemical and bio-chemical transformations that might take place in or on sediments, biodegradation by microorganisms other than bacteria, and biomagnification. We believe that in most cases these omissions will not affect significantly the general conclusions.

Eleven organic chemicals were selected for study. Nine are aromatic compounds typical of those likely to be found in effluent streams from fossil fuel processing plants. These nine compounds are p-cresol, benz[a]anthracene, benzo[a]pyrene, quinoline, 9H-carbazole, 7H-dibenzo[c,g]carbazole, benzo[b]thiophene, dibenzothiophene and benzoquinoline. Two other compounds, methyl parathion and mirex, are pesticides that have been used extensively in field applications where runoff to streams and ponds is likely.

In most cases, the literature data on these compounds were insufficient to allow us to decide which environmental process might be important for detailed study. Therefore, screening studies were conducted to obtain an estimate of the relative importance of each process and measure certain physical properties including solubility and uv spectral absorbance.

Detailed studies were carried out only on those processes which appeared to be competitive in screening studies. Product studies for chemical and microbial transformation processes were included in the detailed studies.

Solution of the equation for first order kinetics, hereafter referred to as the one-compartment model, was used to refine the preliminary assessments using rate constants obtained in detailed studies. This assessment provided a comparision of the relative transformation rates of the chemical under different environmental conditions. The nine-compartment computer model was used to predict in greater detail the transport, distribution, and steady state concentrations in four representative aquatic environments.

ENVIRONMENTAL ASSESSMENT

Objectives and Assumptions

The objectives of the environmental assessment were to:

- Estimate the probable steady concentration of chemicals in the water column, sediment and suspended sediment resulting from continuous low level discharge and to estimate the rate of loss of chemical from these three compartments once discharge has ceased.

- Assess the relative importance of photolysis, hydrolysis, oxidation, volatilization, sorption, and biotransformation in the removal of selected chemicals from solution in natural freshwater systems, exclusive of transfer through food chains or transformation on sediments.

To accomplish these objectives, we made two assumptions that are inherent to the approach taken by this study. These are:

(1) The overall rate of disappearance of a pollutant from solution is controlled only by the dominant transformation and transport processes that were studied separately in the laboratory and by hydraulic and hydrological processes of the aquatic systems.

(2) Each transformation and transport process can be studied independently by laboratory experiments except for hydrologic process, and results of these experiments can be extrapolated to conditions found in natural waters.

One Compartment Model

The one compartment model was used for preliminary assessment of the relative importance of transport and transformation processes and to direct detailed studies for the dominant processes. This model assumes that the system is a single well mixed reactor from which the chemical disappears by transport and transformations by several competitive first order kinetic processes. This model was used to establish priorities for detailed laboratory studies. Because all of the transformation and transport processes are expressed as first order processes, the half life of the chemical toward any process is

$$t_{\frac{1}{2}} = \ln 2/k_i \qquad (1)$$

where k_i is the first order rate constant for any process considered in the model. Since several processes may be competitive additional terms must be added to the denominator of eqn. (1). The net half life of the chemical is

$$t_{\frac{1}{2}} = (\ln 2)/\Sigma_i k_i$$

Assumptions of the One-Compartment Model

- The water body is homogeneous with respect to all physical, chemical, and biological properties.

- Chemical, physical, and biological properties (other than changes in the concentrations of the pollutant and solid masses within the compartments) remain constant.

- The effects of physical, chemical, and biological variables such as temperature, pH, and species composition are included implicitly in the rate factors used in the simulations, but are otherwise excluded.

- Exogenous environmental parameters such as sunlight intensity are constant for a given water body.

- The pollutant is introduced as a pulse at time zero.

- Sorption occurs only between solids and solution and between solution and biological organisms; no sorption occurs between the biota and solids.

- The relative proportions of sorbed and dissolved chemical are those calculated from the partition coefficient between water and a natural sediment; sorption is irreversible and rapid compared to all other transformation and transport processes.

- A portion of the microbial population is acclimated to the specific substrate at all times and the rate of microbial transformation is a function of the number of acclimated microbes and is first order with respect to substrate concentration.

- The microbial yield factor is independent of residence time.

Nine-Compartment Model

The nine-compartment computer model was designed to explore the impact of water body heterogeneity on the transformation and transport processes covered in the laboratory phases of this project. It ignores transformation of sorbed chemicals and accumulation of chemicals within food chains. However, it does permit assessment of the variations in concentrations to which specific segments of food chains will be exposed.

The model contains nine compartments of arbitrary size and composition, which are used to represent segments of the water column or sediments. By selectively removing compartments and appropriately adjusting the constants for water quality and water movement parameters, we can use the model to simulate streams, ponds, or stratified lakes.

Calculations are made on a time-sequenced basis. We assume that flows and mixing occur at the end of each time interval and that volatilization, sorption, and transformations occur within each time interval. A short time interval, generally less than 0.2 hour, is used in the simulations.

The sequence for the time interval Δt is:

(1) Calculate the mass of transformed chemical

(2) Calculate the distribution of chemical between the sediment and aqueous phases, according to the equilibrium partition coefficient.

(3) Calculate the new masses in each compartment based on the assumed inflow-outflow conditions.

The model assumes that the environmental parameters are constant within each compartment but may vary between the nine compartments. Changing parameters, such as day to night, can be approximated by several sequential computer runs in which the output of one run regarding chemical concentration in the daytime is input to the next run where rate factors are modified to represent the nighttime conditions.

An initial concentration of the compound can be arbitrarily assumed in either the solutions, solids or biota in any of the compartments. Loading rates of the chemicals and suspended solids can be input as the specifications of the system.

Environmental Parameters

The constants used to define the volumes of the compartments, the fluxes of materials, and the inflow of chemical at a concentration of 1 $\mu g\ ml^{-1}$ are given in Table 6 (input chemical concentrations are set at 1 $\mu g\ ml^{-1}$ or the solubility limit, which ever is lower). The values used to define the water quality characteristics that directly affect the transformation and transport processes studied in the laboratory are also given in Table 6.

The versions of the model now used are presented schematically in Figure 18. The arrows indicate direction of flows and the rectangles represent the three-dimensional compartments. The model can direct flows between any pair of compartments (for example, from 4 to 8); however, those illustrated are the ones typically used. Compartments 1, 2, and 3 are generally used to represent surface waters; 4, 5, and 6 deep waters; and 7, 8, and 9 sediments. For convenience, inflow was consistently assumed to occur into compartment 1 and outflow from compartment 4.

Laboratory Measurements and Results

The theory and methods used to measure environmental processes important in freshwater aquatic systems are reported in detail elsewhere (Smith et al., 1977).

Laboratory measurements were carried out in two phases. In the first phase screening measurements for six processes and two properties were carried out for preliminary assessment.

Screening studies were designed to measure:

Solubility in water at 20 to 25°C.

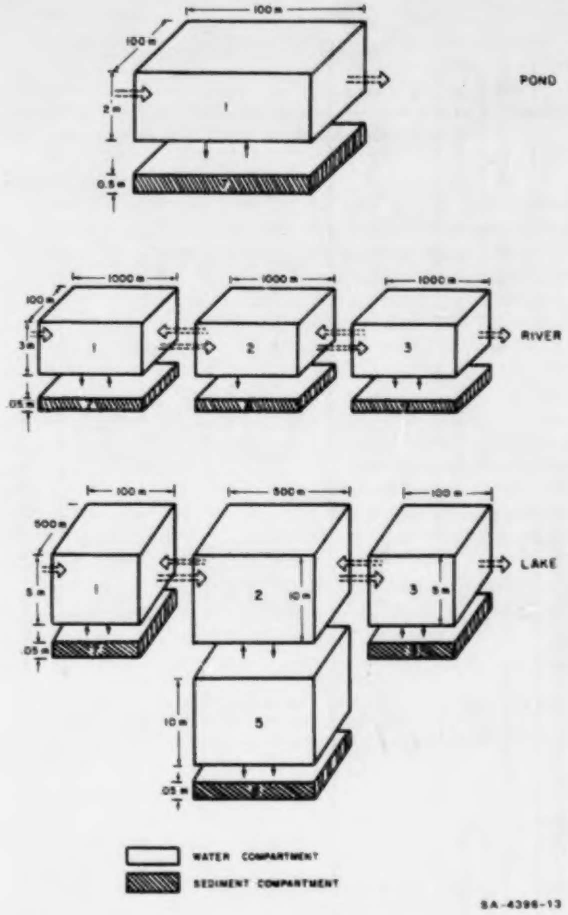

Figure 18. Physical configurations of the pond, river, and lake simulations.

Table 6—Physical Dimensions and Water Quality Characteristics Assumed in the Environmental Analysis

Parameter	Stream	Eutrophic Pond	Eutrophic Lake	Oligotrophic Lake
Physical dimension				
Total water volume (m^3)	9×10^5	2×10^4	5.5×10^6	5.5×10^6
Inflow ($m^3\ hr^{-1}$)	1.0×10^6	0.20	9.7×10^2	9.7×10^2
Mean residence time (hr)	8.3×10^{-1}	1.0×10^4	5.7×10^4	5.7×10^4
Pollutant inflow ($kg\ hr^{-1}$)	1.0×10^3	0.02	0.97	0.87
Water quality				
Total bacteria (cells $m\ell^{-1}$)	10^5	10^6	10^6	10^2
Active bacteria (cells $m\ell^{-1}$)	10^4	10^5	10^5	10
pH	7	8	8	6
Sediment loading ($\mu g\ m\ell^{-1}$)	100	300	50	50
Photolysis activity index[a]	0.5	0.2	0.2	1.0
Oxygen reaeration rate (hr^{-1})	0.04	0.008	0.01	0.01
$[RO_2\cdot]$ (M)	10^{-9}	10^{-9}	10^{-9}	10^{-9}

[a] Factor to account for differences in light transmission through different types of water. Distilled water has an index value of 1.

Table 7.—Summary of Equilibria and Rate Processes at 25°C

	Solubility in water at 22°	Sorption partition coefficient[a]	Volatilization ratio (x 10^3)	Maximum solar photolysis rate constant[b] (sec^{-1})	Photolysis quantum yield	Oxidation rate constant ($M^{-1} sec^{-1}$)	Biodegradation rate constant (ml $cell^{-1} hr^{-1}$)
Rate law	—	—	$\frac{k_V^S}{k_V^O}k_V^O[S]$	$k_p \phi [S]$	$k_p \phi [S]$	$k_{ox}[RO_2\cdot][S]$	$\phi k_{b2}[B][S]$
Chemical							
p-Cresol	1.8[c] mg ml^{-1}	< 10	1.4	6.8×10^{-7}	0.079	20	5.2×10^{-7}
Benz[a]anthracene	5.7 ng ml^{-1}	26,000	1.6	3.9×10^{-4}	3.3×10^{-3}	5,000	0
Benz[a]pyrene	1.2 ng ml^{-1}	76,000	3.6	3.8×10^{-4}	8.9×10^{-4}	1,900	0
Quinoline	6.11[c] mg ml^{-1}	11	2.5	7.6×10^{-7}	3.3×10^{-4}	<3	3.4×10^{-7}
Benzo[f]quinoline	76 μg ml^{-1}	1,300	0.22	1.4×10^{-4}	0.014	<3	1×10^{-7}
Carbazole	1.0 μg ml^{-1}	175	<0.1	2.1×10^{-4}	7.6×10^{-3}	29	5.0×10^{-7}
Dibenzo[c,g]carbazole	63 ng ml^{-1}	18,000	<0.1	5.1×10^{-4}	2.8×10^{-3}	830	0
Benzo[b]thiophene	127 μg ml^{-1}	50	380	9.4×10^{-7}	0.1[e]	83	f
Dibenzothiophene	190 ng ml^{-1}	1,390	120	2.9×10^{-6}	5×10^{-4}	<3	4.95×10^{-7}
Methyl parathion[d]	40[c] μg ml^{-1}	51	<0.06	2.0×10^{-6}	1.7×10^{-4}	<3	2.4×10^{-7}
Mirex	70 pg ml^{-1}	350,000	63	$\sim 4 \times 10^{-8}$	<1.0[e]	<<1	0

[a] Value for a natural sediment collected in Coyote Creek, California.

[b] Maximum rate constant based on June 21 (noon) sunlight hours; divide by two to convert to a calendar day basis.

[c] Literature value.

[d] Rate constant for neutral hydrolysis at 25°C is 9×10^{-8} sec.

[e] Assumed value.

[f] Biodegration was observed only in the presence of naphthalene; there is no kinetic expression that can be used to express this type of biodegradation.

Table 8.—Half-Lives for Transport and Transformation of Chemicals in Simulated One Compartment Eutrophic Lake

	Photolysis[a] half-life (hr)	Oxidation[b] half-life (hr)	Volatilization[c] half-life (hr)	Hydrolysis[d] half-life (hr)	Biodegradation[e] half-life (hr)	Half-life for all processes	Percent sorbed
Chemical							
p-Cresol	4,180	2,900	4.5×10^4	NA	12	12	0.05
Benz[a]anthracene	2.5	160	4.4×10^4	NA	$>10^6$	2.5	55
Benz[a]pyrene	2.5	340	1.9×10^4	NA	$>10^6$	2.5	71
Quinoline	3,000	6×10^4	2.7×10^4	NA	5	5	0.05
Benzo[f]quinoline	7.0	>2,500	3.2×10^5	NA	7	7	6.5
Carbazole	4.6	7,200	$>7 \times 10^5$	NA	30	30	1.0
Dibenzo[c,g]carbazole	1.9	10	$>7 \times 10^5$	NA	$>10^6$	1.9	50
Benzo[b]thiophene	1,020	4×10^4	1.8×10^1	NA	20	20	0.20
Dibenzothiophene	324	2,500	6×10^2	NA	1.4	1.4	6.5
Methyl parathion	480	2,300	$>1.2 \times 10^6$	1,700	30	30	0.25
Mirex	>24,000	$>1 \times 10^5$	1.1×10^3	NA	$>10^6$	2,200	94

[a] Calculated as minimum half-life on June 21 with photolysis index of 0.2($t_{1/2} = 1n2/k_p$ (0.2).

[b] Assume $[RO_2 \cdot] = 10^{-9}$ M in environment.

[c] Assume reaeration rate constant. k_V^O, is 0.01 hr^{-1} in a eutrophic lake.

[d] Half-life calculated at pH 7.

[e] Assumes 10^6 cell ml^{-1} in eutrophic water.

Absorption spectra at wavelengths greater than 290 nm.

Volatilization rates under high turbulence conditions.

Sorption partition coefficients for a Ca-montmorillonite clay, one high organic content natural sediment, and a mixture of four species of bacteria.

Photolysis rates in sunlight and monochromatic light above 300 nm.

Oxidation rates in air saturated water using a free-radical initiator at 50°.

Hydrolysis rates at constant pH and temperature.

Biodegradation rates using enrichment cultures developed from natural waters and aeration effluents from sewage treatment plants that would degrade substrate when it was the sole carbon source.

Preliminary assessment, using a one-compartment model with rate constants based on screening studies, was used to decide which processes should be studied in detail.

For processes that proved to be competitive in preliminary assessment, more detailed laboratory studies were carried out in the second phase to develop kinetic and equilibrium relationships over a broader range of conditions and to analyse for major primary products from chemical and microbial transformations. The results of these measurements on eleven chemicals and their integration in a one-compartment assessment model are summarized in Tables 7 and 8 for a eutrophic lake simulation and synopses of detailed assessment analyses using the nine-compartment model are included on the following pages.

Most of the laboratory data developed for the eleven compounds selected for this study were of relatively high precision; standard deviations for most data were less than ten per cent. Where comparison between theory and measurement was possible as in the case of photolysis, hydrolysis or volatilization, agreement was always within a factor of two, often within twenty per cent. Measurements in natural water or in sunlight were in close agreement with measurements in distilled water or in artificial light. Good agreement was also found for rate constants for microbial transformations of a selected chemical by different methods and using adapted mixed cultures.

Synoposes of Environmental Exposure

The nine-compartment model calculations indicate the concentrations of chemicals in specific compartments of the aquatic systems. To illustrate the kinds of exposure data available from this kind of calculation, we have summarized data from surface and bottom compartments for specific types of water bodies.

p-Cresol

Transformation rates for p-cresol are expected to resemble those found in an eutrophic pond. Hence, even allowing for considerable variability in the importance of photolysis and adsorption as functions of turbidity, water color or temperature, microbial transformations are likely to be the dominant removal mechanism, accounting for at least 90% of the removal of p-cresol from solution.

Steady-state concentrations of p-cresol in solution, on suspended solid and on sediments near point sources in the presence of a continuous discharge of 1 μg ml^{-1} are:

	Solution (μg ml^{-1})	Suspended solids (μg ml^{-1})	Sediments[a] (μg ml^{-1})
Stream	0.99	10	9.78
Pond	0.018	0.17	0.17
Eutrophic Lake	0.039	0.40	0.10
Oligotrophic Lake	0.106	1.04	0.324

[a]This value is averaged for the whole sediment layer.

Quinoline

We can expect that rates for transformation of quinoline will be rapid in all freshwaters, with half-lives ranging from 5 hours or less in warm eutrophic waters to about 25 days in oligotrophic waters. Moreover, unless cometabolism occurs and is significantly affected by concentration at concentrations of 1 ppm or less, the patterns of removal and distribution estimated by our simulations should be generally applicable, as indicated by the similarity of results of, and simulations using, the concentrations of 1 ppm and 10 ppb for quinoline in the in-flows. Photolysis and biodegradation are expected to dominate transformation in oligotrophic and eutrophic waters respectively. Removal should be slowest in the winter, particularly in ice-covered waters where the 25-day half-life may be greatly exceeded due to suppression of photolysis, volatilization. At worst, under thick ice cover, quinoline may not degrade.

Steady-state concentrations of quinoline in solution, on suspended solid and on sediments near point sources in the presence of a continuous discharge of 1 μg ml^{-1} are:

	Solution (μg ml^{-1})	Suspended solids (μg ml^{-1})	Sediments[a] (μg ml^{-1})
Stream	0.99	0	0
Pond	0.007	0	0
Eutrophic Lake	0.022	0	0
Oligotrophic Lake	0.10	0	0

[a]This value is averaged for the whole sediment layer.

Benzo[a]pyrene

The results of the laboratory investigations suggest that benzo[a]pyrene (BaP) will accumulate in the sediment and biota portions of an aquatic environment. A small amount of BaP will be dissolved and will photolyse rapidly.

Several problems were encountered in the course of these studies. Experimental difficulties were encountered because BaP adsorbed on glassware, and its low solubility in water made it difficult to prepare and work with aqueous solutions. We suspect that adsorbed BaP may be transformed by photochemical and chemical oxidation as well as biodegradation processes, but the extent of these processes could not be evaluated by our laboratory procedures. Finally, there is literature evidence that biodegradation of BaP may take place under different conditions and with different organisms than those used in these studies. Therefore, the general conclusion is that BaP will accumulate in sediments and microorganisms and may degrade slowly in sediments with half-lives of the order of a few years.

The nine-compartment model predicts that the steady-state concentrations of benzo[a]pyrene in solution, on suspended solids and on sediments near point sources in the presence of a continuous discharge of 1 ng ml^{-1} are expected to be:

	Solution (ng ml^{-1})	Suspended solids (ng ml^{-1})	Sediments[a] (ng ml^{-1})
Stream	1.5×10^{-3}	7.4	9.9
Pond	1.5×10^{-6}	0.07	0.07
Eutrophic Lake	1.5×10^{-5}	0.54	0.14
Oligotrophic Lake	1.9×10^{-6}	0.093	0.023

[a] This value is averaged for the whole sediment layer.

9H-Carbazole

The results of the laboratory investigation suggest that 9H-carbazole should not be a significant environmental hazard. Both photolysis and biodegradation are rapid. Carbazole is not sorbed strongly, and it will not accumulate in either sediments or biota. The volatilization and oxidation rates are slow. The photolysis transformation products appear to be coupling products containing one or two oxygen atoms, but their structures were not positively identified. No biodegradation products were observed, and it is likely that complete degradation occurs.

The nine-compartment environmental exposure model predicted that the steady-state concentrations of carbazole in solution, suspended solids, and sediments near point sources in the presence of a continuous discharge of 1 μg mℓ^{-1} (1 ppm) carbazole are expected to be:

	Solution (μg mℓ^{-1})	Suspended solids (μg g^{-1})	Sediments[a] (μg g^{-1})
Stream	9.3×10^{-1}	190	180
Pond	1.3×10^{-2}	2.5	2.5
Eutrophic Lake	3.1×10^{-2}	6.2	0.88
Oligotrophic Lake	4.0×10^{-2}	8.0	1.2

[a] This value is averaged for the whole sediment layer.

7H-Dibenzo[c,g]carbazole

The results of the laboratory investigations suggest that 99% of the 7H-dibenzo[c,g]carbazole (DBC) entering natural waters will be sorbed onto solids and that the remaining 1% will be rapidly transformed photochemically to yield two products, one of which has added oxygen from reaction with water and is probably phenolic. Photolysis of DBC is expected to be the dominant process in clear waters. In turbid waters, sorption by suspended sediments will be more important. No information was obtained concerning possible phototransformation of DBC sorbed on suspended sediments. Enrichment cultures that could degrade DBC were not obtained. Volatilization of DBC will be extremely slow.

Steady-state concentrations of DBC in solution, suspended solids, and sediments near point sources in the presence of a continuous discharge of 10 ng ml^{-1} (10 ppb) are expected to be:

	Solution (ng ml^{-1})	Suspended solids (ng g^{-1})	Sediments[a] (ng g^{-1})
Stream	2.8	55,000	49,000
Pond	0.01	18	200
Eutrophic Lake	2.9	580	81
Oligotrophic Lake	1.5×10^{-3}	30	4.4

[a] This value is averaged for the whole sediment layer.

Benzo[b]thiophene

The results of the laboratory investigations suggest that benzo[b]thiophene (BT) is not likely to accumulate in an aquatic environment and that volatilization is the major environmental fate of BT in aquatic systems. However, under some conditions, such as a deep lake where volatilization is slow because the turbulence is also low, photolysis and perhaps biodegradation may be significant.

The laboratory procedures used to estimate volatilization, photolysis, oxidation rates, and the partition coefficients on a natural sediment and on a mixture of microorganisms were satisfactorily applied to BT. Biodegradation was a problem because BT degradation occurred only in the presence of naphthalene by what appeared to be a co-metabolic process. Because adaptation to BT required twice as long as for quinoline we used kinetic constants one-half as large as those used for quinoline. While we recognize that this choice is somewhat arbitrary, the constants selected probably represent upper bounds for the environmental biodegradation rate.

The nine-compartment environmental exposure model predicted that the steady-state concentrations of benzothiophene in solution, suspended solids, and sediments near point sources in the presence of a continuous discharge of 1 μg ml^{-1} (1 ppm) benzothiophene are expected to be:

	Solution (μg ml^{-1})	Suspended solids (μg ml^{-1})	Sediments[a] (μg ml^{-1})
Stream	0.93	46	44
Pond	0.0089	0.43	0.45
Eutrophic Lake	0.023	1.2	0.30
Oligotrophic Lake	0.031	1.6	0.41

[a] This value is averaged for the whole sediment layer.

Methylparathion

The major influx of MP into natural waters should occur shortly after application either as sprays, drift, or run-off, possibly with a large fraction adsorbed to soil particles. If so, the degradation rates should most nearly resemble the pond simulation, which assumes large populations of metabolically active microbes and high turbidity. The much smaller influx of MP that can be expected in winter, when biodegradation rates are suppressed, probably also enters mainly in the adsorbed form. If so, most of the MP is probably removed from the water column by sedimentation before undergoing substantial degradation, and then degrades slowly by hydrolysis or biodegradation. However, the fraction that enters as a solution during the winter months probably undergoes moderate degradation by photolysis and hydrolysis before being transported to the sediments. If so, degradation in eutrophic waters during the winter should roughly approximate the rates for compartment 1 of the oligotrophic lake simulation, which shows the maximum contribution of abiotic degradation mechanisms.

Given these assumptions, biodegradation should account for roughly 1000 times more degradation of MP in actual waters than any other form of degradation, and most of the degradation should occur in the summer.

The nine-compartment environmental exposure model predicted that the steady-state concentrations of methylparathion in solution, suspended solids, and sediments near point sources in the presence of a continuous discharge of 1 μg ml^{-1} (1ppm) methylparathion are expected to be:

	Solution (μg ml^{-1})	Suspended solids (μg ml^{-1})	Sediments[a] (μg ml^{-1})
Stream	0.99	49.5	49.1
Pond	0.012	0.60	0.60
Eutrophic Lake	0.06	3.0	0.51
Oligotrophic Lake	0.098	4.9	1.4

[a] This value is averaged for the whole sediment layer.

Environmental Persistence

In addition to information on concentrations and distribution of a chemical in different parts of a water body, data on rates and equilibria also can be used to calculate the time required for a chemical to reach a steady-state concentration in water or sediment under constant discharge and to calculate the persistence of the chemical when the discharge is stopped. Figures 19 and 20 illustrate the cases of benzo[a]pyrene (BaP) and benzo[b]thiophene (BT). The patterns for the chemicals are similar: concentrations of chemical in the suspended solid and in the water column follow similar courses because of the rapid equilibration of the two phases; the steady-state concentration in sediment is reached much more slowly and remains high long after discharge has stopped because transport to and from the sediment layer is relatively slow. Moreover the persistence of the chemical in the water column is much longer than expected from a simple one compartment analysis of the data although the initial concentrations drop by factors of 10 or 10,000.

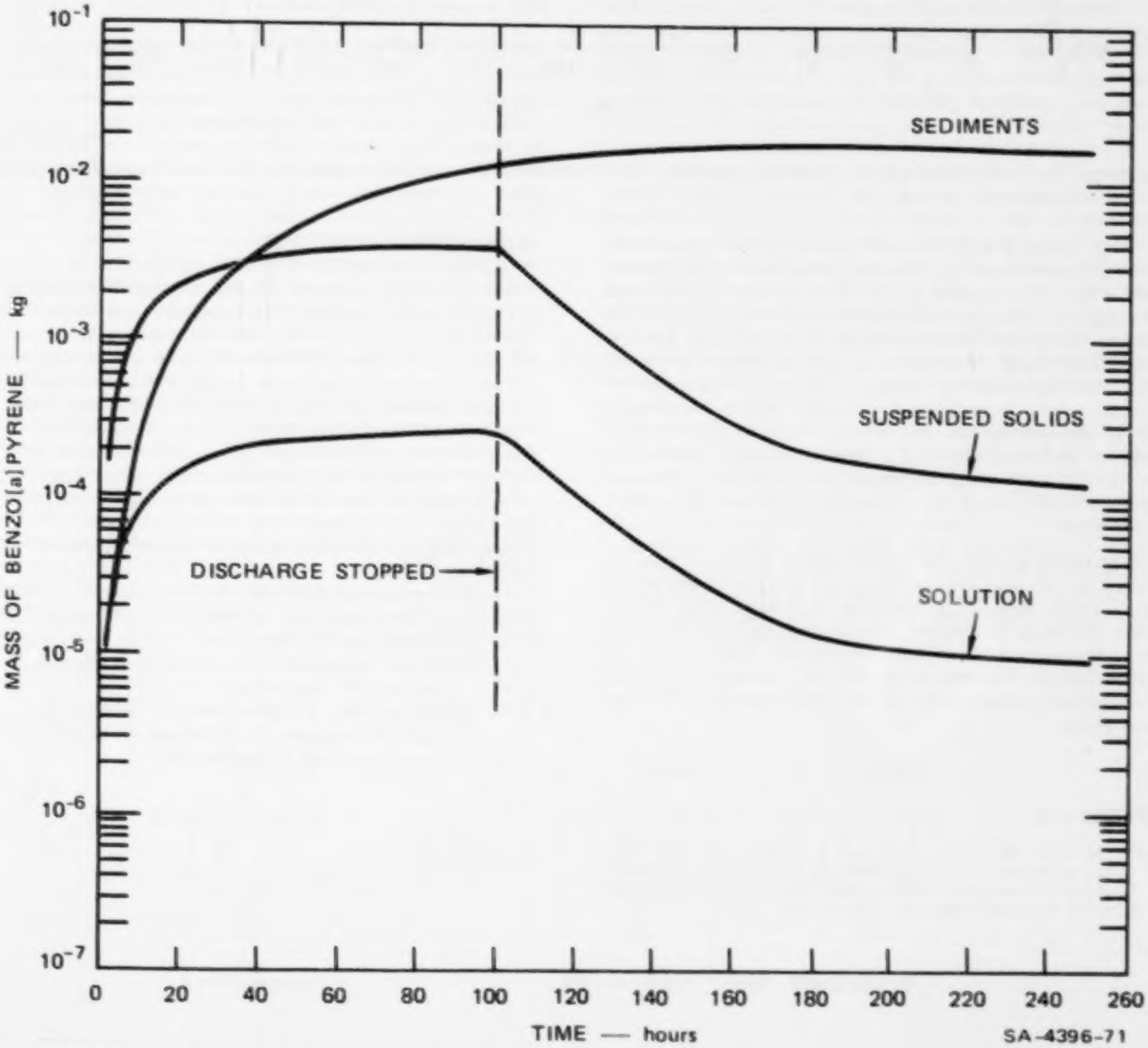

Figure 19. Persistence of Benzo[a]pyrene in a partially mixed two-compartment system (pond). Continuous discharge of 2 × 10^{-5} kg of benzo[a]pyrene per hour.

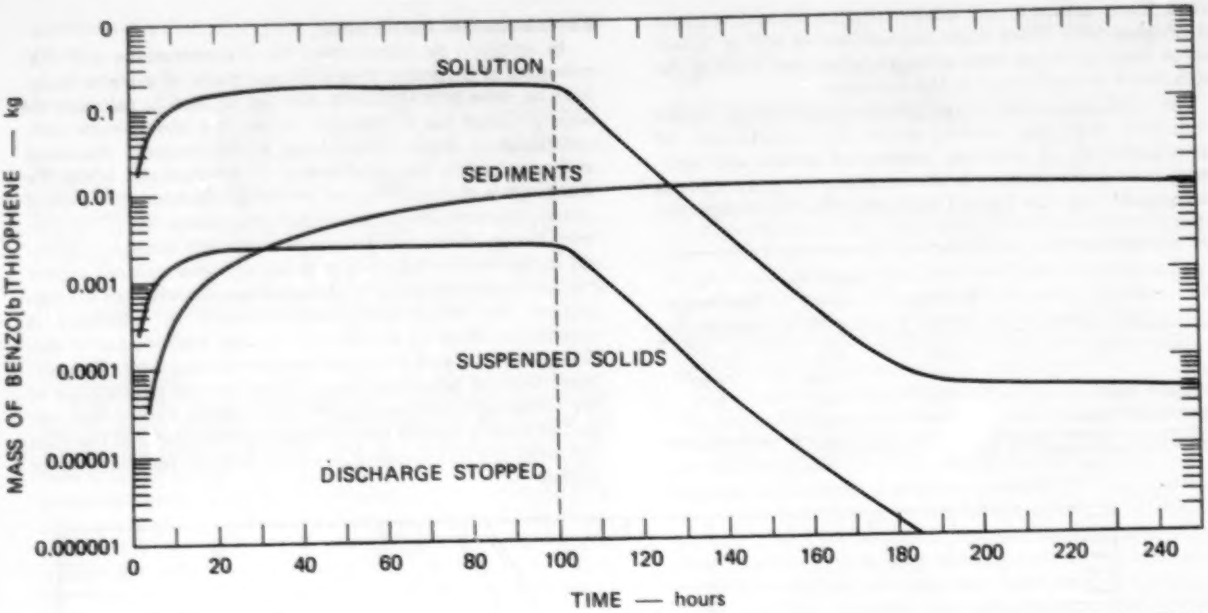

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Figure 20. Persistence of benzo[b]thiophene in a partially mixed two-compartment system (pond). Continuous discharge of 0.02 kg of benzo[b]thiophene per hour.

Both the lag in attainment of the steady-state concentrations in the sediment and the long persistence of the chemicals in the water column reflect the slow rates of exchange of chemicals between sediment and the water column; in this model the half-life for scouring of the sediment phase is about 1700 hours. Thus sediments act as buffers to restore the concentrations of chemicals lost by transport or transformation from the water column. Where the proportion of chemical sorbed to sediment is high, as in the case of BaP, the buffering effect of sediment strongly inhibits the loss of BaP from water by photolysis resulting in a persistent level of BaP only 20 times as small as the original concentration.

Compared to BaP, the much smaller proportion of BT sorbed to sediment results in a much smaller buffer effect and a much more dramatic drop in concentration of BT when discharge is stopped even though BaP and BT have about the same net half-lives towards all processes in the water column. However, even with BT, sediment buffering is important in maintaining a low concentration of BT for long times.

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Biodegradation and Environmental Metabolism of Organic Pollutants

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My discussion will focus on some of the problems in the area of terrestrial microcosms illustrating these areas with the data I am most familiar with, our own. I shall endeavor to show you some of the difficulties one might encounter as one approaches the problem of testing chemicals as they are introduced deliberately or inadvertently into a soil ecosystem.

One of the major problems is semantics. One should distinguish between partial transformations which do not lead to inorganic end-products, and complete degradation (which is termed mineralization) that leads to carbon dioxide, water and often chloride, ammonium, nitrate or orthophosphate. There is a perennial difficulty with the term biodegradation because it has been used so many different ways. For some people, biodegradation refers solely to the destruction of the parent molecule. For others, it refers to the loss of a functional group (i.e., chloride, ammonium or nitrate), the loss of absorbency, or the formation of water-soluble substances from lipid-soluble compounds. There are several dangers in considering that such "biodegradation" is environmentally safe, as will be discussed subsequently.

Biodegradation also refers to complete mineralization in which there are no environmental hazards produced except as we may consider CO_2 or increasing eutrophication associated with nitrate pollution to be an environmental hazard. Potentially toxic organic products do not remain.

As a rule, photochemical, non-light induced chemical reactions, and plant or animal metabolism of organic chemicals do not lead to mineralization. I do not know of a single instance where ambient light or chemical substances in natural environments totally destroy a complex organic material and convert it to carbon dioxide and water. Ultimate degradation or mineralization requires microbial intervention. In partial transformation, the organic product that accumulates may have an effect on the target species (if it is a pesticide) or on an entirely different organism. A good example exists in the literature on phytotoxins. Pentachlorobenzyl alcohol was introduced in Japan a number of years ago as a non-phytotoxic antifungal agent to control rice-blast disease. Presumably because of the chemical's lack of phytotoxicity, it was not a serious environmental problem. Pentachlorobenzyl alcohol, however, was converted in soil to penta-, tetra-, and trichlorobenzoic acids (Figure 21). These compounds are both phytotoxic and persistent. Thus, although the parent molecule has disappeared (some individuals would say it was "biodegraded"), the parent substance, which is not a hazard to crop and noncrop plants, is converted to a substance which has a severe effect on a variety of plant species (Ishida, 1972). Hence, although the original chemical problem was gone, the pollution load in the soil was not overcome. So, the semantic issue is a real one, and the matter cannot be ignored. The ultimate fate of the molecule and the identity of potential and persistent intermediates must be defined prior to concluding that no significant environmental problem exists.

Some of our recent work has been associated with another difficulty related to the accumulation of intermediates and their reactions in soil and, in some instances, water. The issue: nitrosamines. It is well known that nitrosamines are carcinogenic, mutagenic, and teratogenic. In addition, studies show that some are persistent, some leach through soil so that they can enter the groundwater supply, and some can be taken up by economic species of plants so they may appear in the food supply (Dean-Raymond and Alexander, 1976).

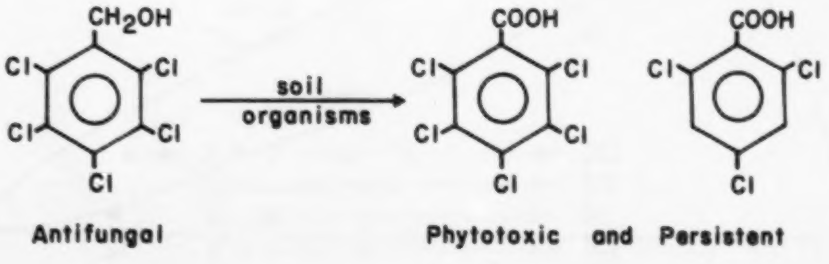

Figure 21. Change of pesticide action spectrum from a fungicide applied to soil (Ishida, 1972).

The molecule has two kinds of precursors that join together to give a nitrosamine. One category of precursor is the secondary amine. Secondary amines are found in plants, algae, fish, food, pesticides, and some industrial chemicals. Secondary amines can also be formed from tertiary amines, which are found in fish and in pesticide preparations. Quaternary compounds undoubtedly also give rise to secondary amines. Therefore, this precursor of nitrosamines is common in soil and water.

The second precursor is nitrite. Nitrite, except as added to foods, is not a common constituent in environments or products to which humans are exposed, but it is produced and may reach high enough concentrations in soil to lead to nitrosation reactions.

Figure 22 shows a simple reaction that gives rise to dimethylnitrosamine. Nitrosamines can have longer alkyl chains or aromatic portions. The reaction shown can occur in soil to which is added dimethylamine, a simple, nontoxic compound (Ayanaba and Alexander, 1974; Ayanaba, Verstraete, and Alexander, 1973). Nitrate is reduced in soils to nitrite. Nitrite will also accumulate in soil that receives high levels of ammonium fertilizers and are either alkaline initially or reach a high pH because of the hydrolysis of urea or decomposition of organic nitrogen in the fertilizer. Although the process can be microbial (Ayanaba and Alexander, 1973), our recent data indicate that nitration in soil may be nonenzymatic (Mills and Alexander, 1971).

Our early data on the formation of dimethylnitrosamine (DMNA) from dimethylamine (DMA) as shown in Figure 23 illustrate that dimethylamine disappears. When nitrite is present, dimethylnitrosamine appears; subsequent studies showed persistence of the nitrosamine (Tate and Alexander, 1975). These data serve as an illustration of one of the major reasons to study environmental metabolism: a nontoxic compound, dimethylamine is transformed to a toxic substance, dimethylnitrosamine. A second reason, not shown in these data, is that the precursors may be readily biodegraded (that is, they are mineralizable), whereas the product may be persistent. A third reason is that a toxicant for one species; (such as a pesticide) may be converted to a toxicant for an entirely different species; for example, the fungicide thiram can be converted to dimethylnitrosamine—a human carcinogen. For these reasons, it is essential to establish what happens to a compound in soil or in

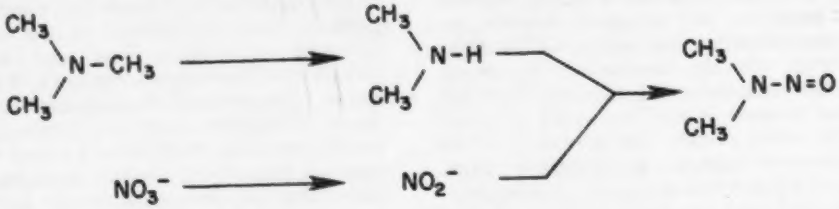

Figure 22. Microbial reactions leading to nitrosamine formation.

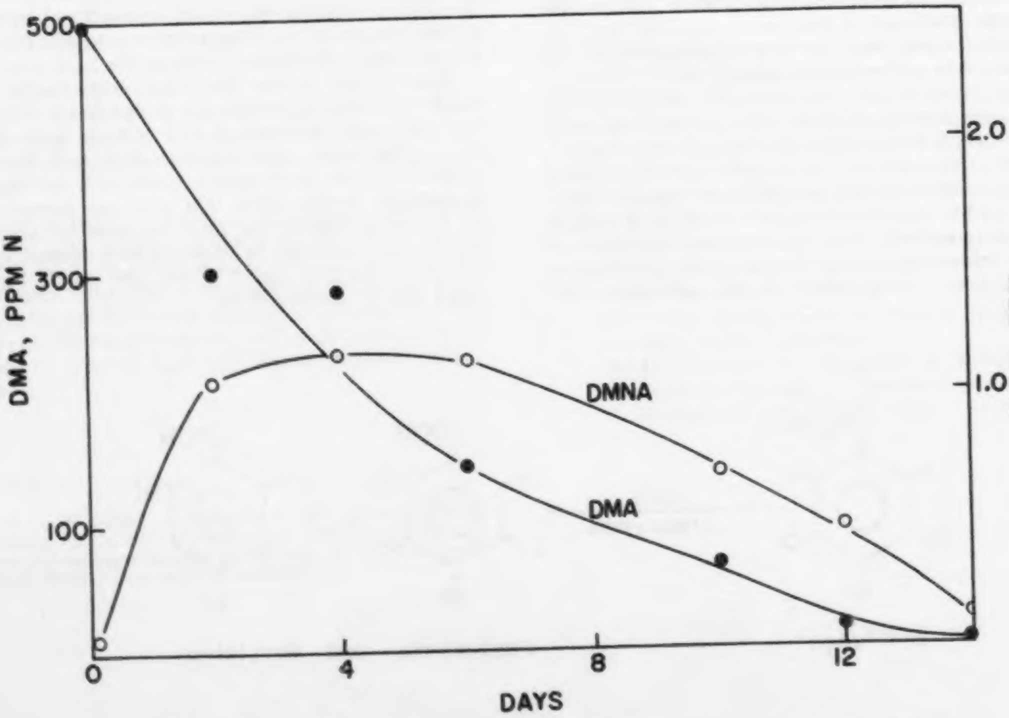

Figure 23. Formation of dimethylnitrosamine (DMNA) in an environmental sample amended with dimethylamine (DMA).

model terrestrial communities, not simply the fate of the parent molecule but also the appearance of ecologically important intermediates.

Another topic that must be considered is how these studies should be conducted. Several unique difficulties should be considered in planning investigations of these sorts. First, the concentration of the pollutant is minute, although biochemists characteristically work with substrates at very high concentrations. Second, a sample of soil contains hundreds of populations, yet microbiologists like to work with pure cultures. Third, most of the organic compounds of current concern and of likely importance in the future are exotic. Hence, the biochemical literature will not contain the metabolic pathway, and the investigator will have to establish the pathway himself. Fourth, the environment is extremely complex, and the colloidal organic materials, variable quantities and types of clay, and water relationships could create major difficulties for the unwary. For these reasons, experimental models are commonly used. The pathway should be defined first, at least in broad outline, and its validity established in the soil microcosm. Moreover, it is convenient to use a single species in pure culture before seeking pathways in the soil community with its many dissimilar populations.

Even with the pure culture, a solution provided with high substrate concentrations rather than the diverse community in soil provided with low substrate levels, the investigation is not simple. Consider for example, the biodegradable herbicide 2,4-D (Figure 24). Each compound in the sequence which is excreted by the microbial cell is of potential importance. Given this metabolic pathway, what happens in nature or at least in the terrestrial microcosm? Providing answers now is not too difficult, a sequence has been established and products have been identified.

Another question to bear in mind: what is the basis for assuming that only one model applies in nature? Is the well defined cultural model representative of what transpires in nature? In Table 9 are data that illustrate the question. The

Table 9—Beta Oxidation in Soil

Substrate	Products
PhO-C_4	PhO-C_2
PhO-C_6, PhO-C_8, PhO-C_{10}	PhO-C_4, PhO-C_2
PhO-C_5	PhO-C_3, PhOH
PhO-C_7,PhO-C_9, PhO-C_{11}	PhO-C_5, PhO-C_3, PhOH

PhO: 2,4-dichlorophenoxy

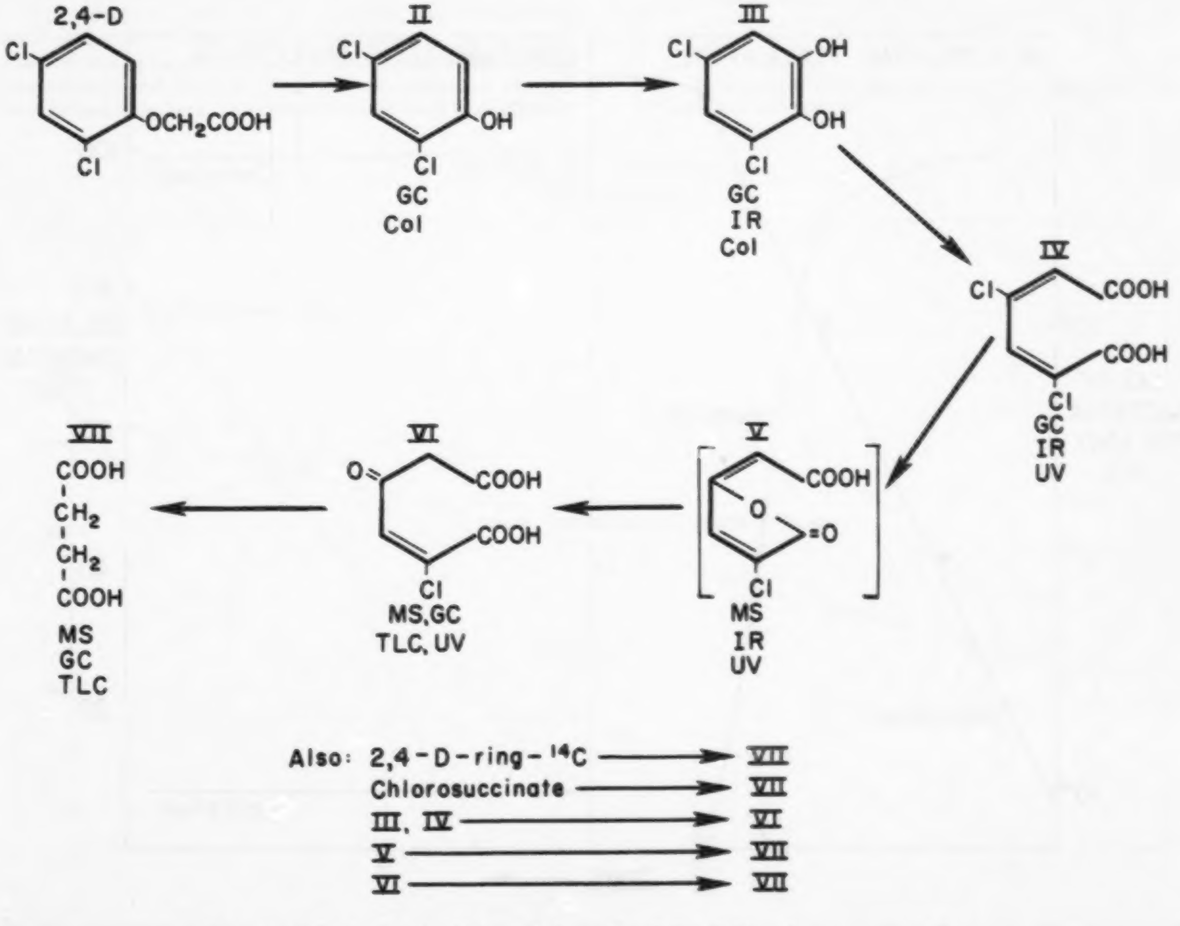

Figure 24. Steps in the microbial metabolism of 2,4-D (2,4-dichlorophenoxyacetic acid).

results came from investigations of phenoxy herbicides. PhO is the dichlorophenoxy moiety of the molecule and C3 to C8 represent three-to-eight-carbon fatty acids. Part of the work was done at Wye College (Taylor and Wain, 1962), part at Cornell (MacRae and Alexander, 1963). In one instance the microorganisms cleaved the side chains by a sequence known as beta-oxidation, removing two carbons at a time from the end of the chain. In the second instance, the microorganisms catalyzed a cleavage at the ether linkage to yield the fatty acid and dichlorophenol. Given these two models, one can test soil directly. The results of such an inquiry are summarized in Table 10 (Gutenmann et al., 1964). These data suggest that only one of the two mechanisms is applicable to this soil.

Table 10—Metabolism of Phenoxyalkanoates

	Products	
Substrate	Nocardia	Flavobacterium
PhO-C_3	—	C_3
PhO-C_4	PhO-C_2	C_4, PhOH
PhO-C_5	PhO-C_3	C_5
PhO-C_6	PhO-C_4, PhO-C_2	C_6
PhO-C_7	PhO-C_3	C_7, C_8
PhO-C_8	—	C_8, C_6

PhO: 2,4-dichlorophenoxy

If there are two possible pathways based on the tests in culture, could there be five or even 20 or 30? The answer probably is no, because biochemical evolution seems to have given rise to only a few pathways—sometimes more than 1, but rarely more than three or four.

Another problem is cometabolism, the metabolism of a compound by a microorganism which cannot use that compound as a nutrient. It has often been observed that chlorinated hydrocarbons and a variety of other pesticides and industrial chemicals are transformed in soil, but the reaction ceases if the soil is sterilized. However, no microorganisms able to grow on the compound can be isolated. For example, DDT is transformed in soil biologically, but an organism able to grow on it has yet to be obtained.

A compound subject to cometabolism presents a special situation. Consider the existence of a mineralizing bacterial population which grows on a particular chemical and is present at an initial density of 10^4 per gram. These bacteria grow at the expense of the chemical, at the same time, the chemical disappears in proportion to the rise in cell density (Figure 25). If a second increment of the chemical is added to the soil, it will disappear even more readily because of the many active bacteria present. On the other hand, if an organism does not grow on but will cometabolize that particular compound, then its abundance will remain constant with time. This bacterium uses some other organic nutrient

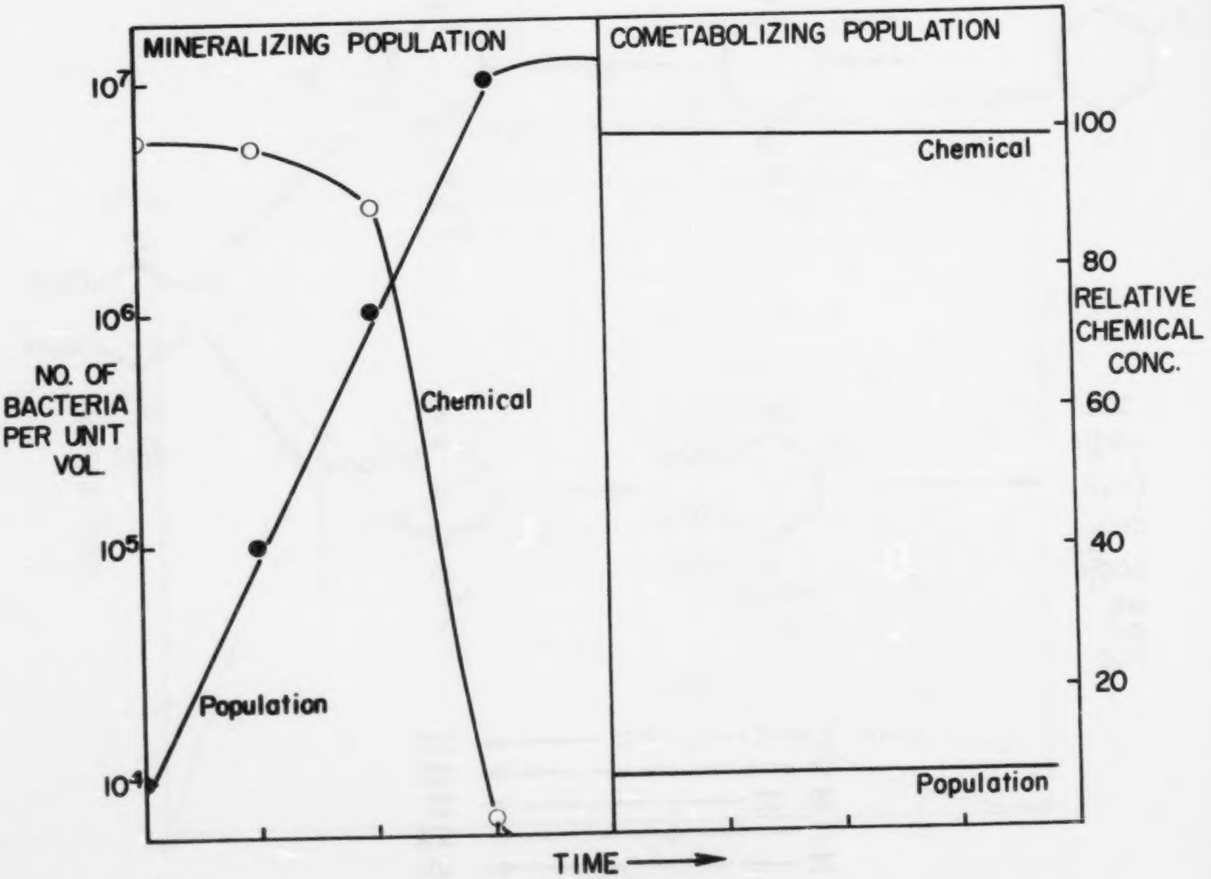

Figure 25. A comparison of changes in bacterial mineralization and cometabolism of an organic compound.

in the soil; hence, an acceleration in the decomposition rate of the chemical does not occur since the bacterial numbers remain small. An obvious consequence of such a concept of cometabolism is persistence of the pollutant in question. Furthermore, products should accumulate according to this view of cometabolism because the responsible organisms are unable to metabolize the substrate extensively; if they could, the compound would likely serve as a carbon and energy source and hence would sustain growth.

Another potential problem is the facile extrapolation that many investigators make between pure culture or biochemical studies in the laboratory and what happens in nature. In pure culture work, cells of one organism are present; in soil, hundreds of different species exist. In culture 10^9 to 10^{10} cells per milliliter are common, but such numbers do not exist in soil. Moreover, protozoa may destroy a large percentage of the bacteria in soil, but this does not happen in culture because protozoa are not present. Furthermore, a particular metabolic sequence may not occur although it may be inducible in the laboratory.

Another problem: all soils are not the same. Soils differ enormously, and there is no such thing as a standard soil. Soils thus differ in the types and amounts of clay, and clays have a marked influence on the rate of decomposition. Figure 26 shows how clays slow the decomposition of decylamine, even in a simple medium (P. Wszolek and M. Alexander, unpublished data).

The physical presence of particles also influences mineralization. Clays have a diameter of about two micrometers or less, silts have diameters from about 2 to 50 micrometers and sand particles possess diameters greater than 100 micrometers. The pores between such particles are about 1/10 the size of the particle itself. Such pores are quite small, therefore, and one is forced to ask how is an organism going to get into a particular mocropore where the chemical is deposited. The possibility of minute particles retarding the decomposition, merely because of the small pores between them, is suggested by the data in Figure 27. In parti-

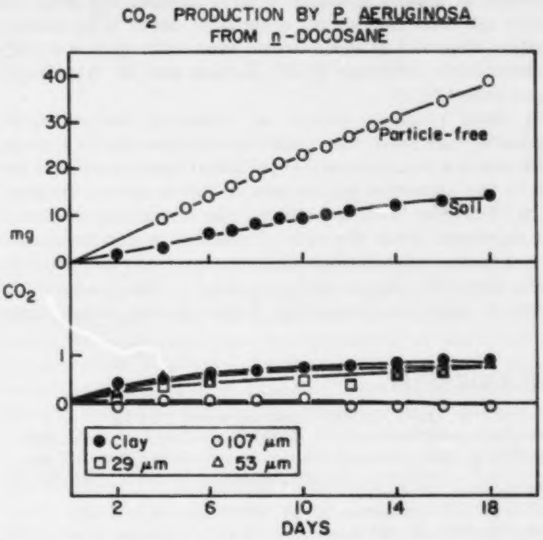

Figure 26. Degradation of free and clay-bound decylamine by a microbial enrichment.

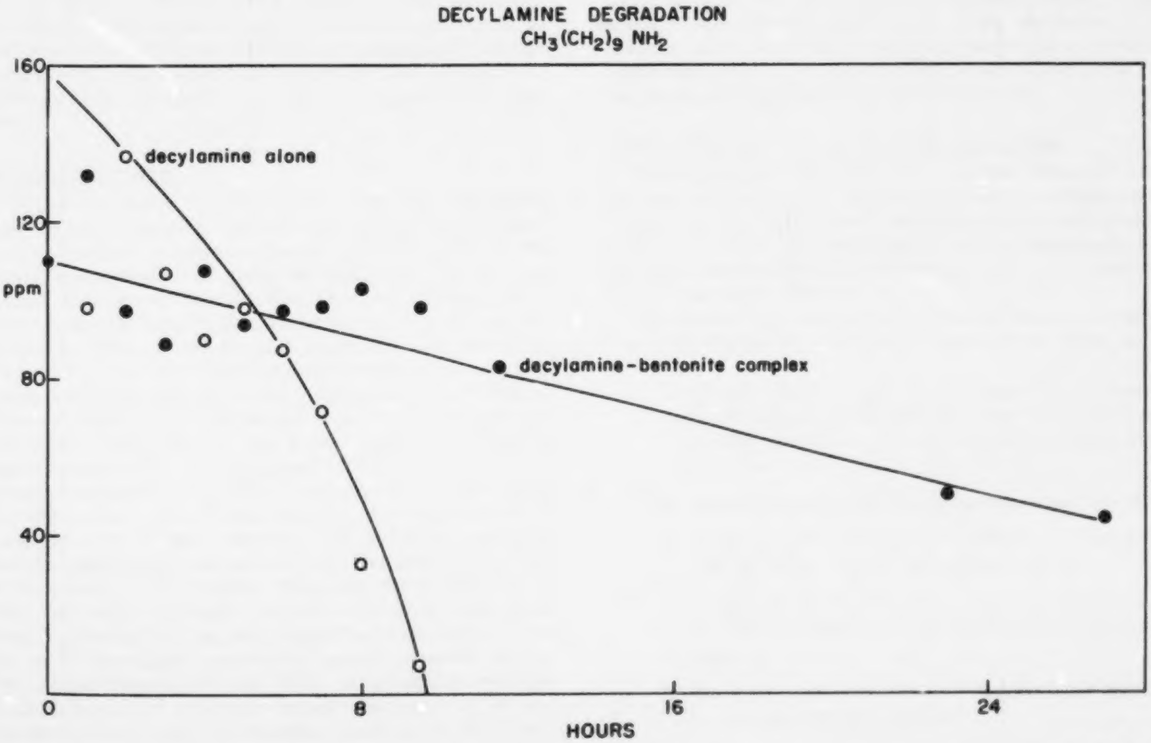

Figure 27. Formation of CO_2 in the decomposition of n-docosane by *Pseudomonas aeruginosa* in the presence of soil, clay, glass beads of three sizes and in particle-free solution.

cle-free solutions, the substrate undergoes rapid decomposition. In the presence of the small particles (glass beads) by contrast, the rate of attack is markedly reduced (J. Haines and M. Alexander, unpublished data).

Excessive soil moisture has a very dramatic effect. Even a simple sugar may be persistent when soils are under anaerobiosis for some time. Thus, when radioactive glucose is added at depths of 1.5, 7, and 12 centimeters to a soil which has been flooded for some time, there is no decomposition observed at some depth, even with such a readily biodegradable substrate (O.W. Kilham and M. Alexander, unpublished data).

A final problem relates to substrate concentration. Typically, high substrate concentrations are used in models, but is the rate in nature at low pollutant levels similar to the rate in the terrestrial microcosm at higher concentrations? From Michaelis-Menten kinetics, one would expect that at low substrate levels the rate of reaction would be slower than at high concentrations. Still, the reaction rate would be finite. However, microorganisms need a certain amount of energy to maintain themselves. If their energy requirement for maintenance is not satisfied, microorganisms will not develop; if they do not develop, there will be no destruction of a particular compound. It is thus plausible to believe that when a substrate is present at very low concentrations, its rate of diffusion to the cell may be insufficient to maintain the organisms, and the chemical will then persist. Consider again the case of a bacterium with radioactive glucose. The data from such a study suggest that this readily usable molecule may be essentially nonbiodegradable when present in solution at the part-per-trillion level (R.S. Boethling and M. Alexander, unpublished data). These preliminary observations point to a serious problem if the same occurs with heterogeneous microbial communities in soils or waters.

These examples hopefully show some of the problems in dealing with biodegradation and environmental metabolism of organic pollutants. The problems are not insurmountable, however, and the importance of the work and the need for reliable information should prompt a more thorough characterization of how soil communities modify pollutants to the benefit, but sometimes to the detriment, of mankind.

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Approaches to Investigating Soil Degradation and Dissipation of Pesticides

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ABSTRACT

Soil and/or sediment are considered the ultimate repository of all chemicals deliberately released or which escape into the environment. Knowledge of the factors affecting the dissipation of chemicals in soil is a critical aspect of understanding their environmental fate, behavior and impact. What happens to the chemical in soil or sediments may ultimately determine where else in the environment it will be found. Thus, detailed information regarding its toxicology, metabolism, chemical reactions, sorption, volatilization, movement, cumulation and persistence are essential to continue environmentally acceptable uses and disposition of chemicals. The purpose of this presentation is to examine some of the methods used to assess the degradation and dissipation of pesticides in or from soils. Such methods as soil perfusion, biometry, and biological and chemical assays each contribute information useful to assessing the behavior patterns of chemicals in soil. These methods will be discussed with respect to their advantages, disadvantages, relevance, and types of information produced.

INTRODUCTION

Millions of acres of soil receive, each year, substantial quantities of chemicals together with subsidiary materials such as carriers, solvents, diluents and synergists. If the natural disappearance of these chemicals from soil does not exceed their rate of introduction, they will accumulate in soil and contribute to the contamination of the environment (Hamaker, 1972). From the soil, chemicals can move into the water, the air, and the food crops grown in the soil. It is clearly necessary that we control the quantity and kind of chemicals introduced into the soil so as to avoid accumulation. Fortunately, the soil has a great capacity to degrade organic compounds as is evidenced by the disappearance of the previous year's vegetation. In fact, if we had been using soil rather than water for the disposal of sewage and other wastes as done in some countries, we probably would not have the problem of pollution of our lakes and streams that we now face in this country (Hamaker, 1972). While it is true, that some synthetic organic chemicals, and some natural products may be less "digestible" than others, even the most intrasigent chemicals appear to yield to the degradation processes in soil. With our current knowledge of the behavior of synthetic organic chemicals in the environment, they need not become a problem at all if their rate of introduction and dissipation is managed properly.

Three main types of pesticide transformation are known to occur in soil: photochemical; chemical; and microbiological. The investigation of these phenomena under field and laboratory conditions contributes to a significant part of our understanding of the environmental fate and behavior of pesticides. Although photodecomposition of pesticides in air and water is a very common occurence, little photodecomposition in soil is expected since radiant energy is strongly sorbed by soil and therefore relatively unavailable for photochemical degradation. Thus laboratory-scale soil degradation experiments are conducted in the dark to eliminate photolysis as a possible significant factor in these experiments. Both qualitative and quantitative pesticide degradation studies in soil are necessary. Investigations of the aerobic and anaerobic degradation of pesticides in soil under laboratory conditions establishes primarily the degradation pattern of the pesticide in soil and how it may be affected by various environmental parameters. A field study which involves application of the pesticide in the manner in which it is to be used, also takes into account the additional influence of light and/or plants on the pattern of the pesticide degradation in the environment.

APPROACHES TO SOIL METABOLISM

Microbiological degradation is a critical factor affecting the fate and behavior of chemicals in soil (Kaufman, 1974). Whether or not a chemical is persistent, short-lived, mobile, stationary, sorbed, activated, inactivated, or eventually constitutes a residue problem may depend upon its metabolism by soil microorganisms.

Soil metabolism studies are most efficiently conducted with radiolabeled pesticides and should be designed to:

1. Characterize the rate of breakdown of the parent pesticide and products so that their stability in soil can be classified and related to reference compounds;
2. Determine products and degradation pathways;
3. Develop some concept of variability in breakdown rate as related to soil and climatic factors;
4. Determine the influence of soil and climatic factors on accumulation of different soil products; and
5. Maintain a balance sheet of radioactivity applied to the experimental sample.

A wide variety of approaches have been used to examine pesticide metabolism in soil under laboratory conditions.

Basically, they fall into a limited number of categories. These basic systems include soil perfusion systems, biometers, gas flow-through systems, and simple shake cultures. Selection of any single method, of course, has depended on several factors such as, the experimental objective, expertise of the investigator, availability and/or cost of the necessary equipment, and to some extent the nature of the chemicals being investigated.

Soil Perfusion

Soil perfusion devices have been developed to study soil microbiological processes such as nitrification, denitrification, pesticide degradation, and microbial utilization of water-insoluble substrates such as sulfur or coal (Audus, 1946; Lees and Quastel, 1946; Chase, 1946; Lees, 1947; Temple, 1951; Sperber and Sykes, 1964; Kaufman, 1966; Goswami and Green, 1971). The design shown in Figure 28 is equally adaptable to either positive or negative pressure systems. It has been used successfully with CO traps both preceding and succeeding the system. Its principle advantages are: (a) adaptability to use with either positive or negative pressure systems; (b) ease of maintaining sterility if desired; (c) ease of sampling perfusate; (d) ease of controlling rate of perfusion; (e) adaptable to use with gases of known composition; (f) infrequent adjustment requirements; (g) self-supporting and compact design; and (h) relatively inexpensive parts.

This system has been used successfully to observe microbial degradation of pesticides (Kaufman and Kearney, 1965; Kaufman et al, 1970; Kaufman, 1977). In such investigations, soil containing a pesticide was perfused with distilled water; or untreated soils were perfused with aqueous solutions of pesticides. Under these conditions degradation was determined either by measuring the rate of pesticide disappearance, or the accumulation of breakdown products, or both. The kinetics involved with the degradation of a pesticide in similar systems have been discussed elsewhere (Audus, 1960). Typically, a lag period occurs during which there is no change in pesticide concentration, followed by a period of rapid disappearance of the pesticide (Figure 29). Subsequent additions of the pesticide decompose more rapidly than the first. This period of rapid breakdown is generally accompanied by proliferation of "effective" microorganisms capable of utilizing the pesticide as a sole or supplemental source of energy and nutrient. Soils in which this phenomenon has occurred are frequently described as "enriched" soils (Kaufman and Kearney, 1976). Isolation of pure cultures of effective organisms from enriched soils has been achieved via dilution plate techniques (Kaufman and Kearney, 1965).

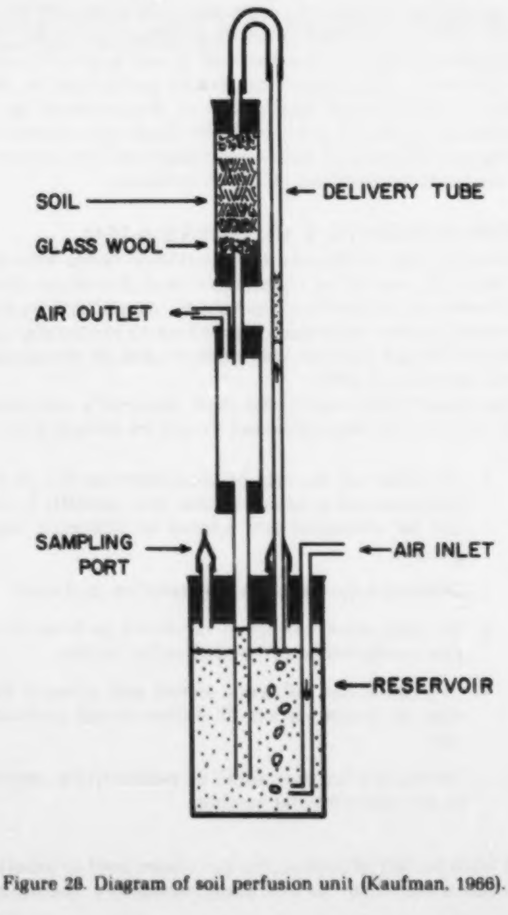

Figure 28. Diagram of soil perfusion unit (Kaufman, 1966).

Data obtained with soil perfusion units have been primarily useful in assessing the relative biodegradability of pesticides (Kaufman, 1966, 1977); factors affecting microbial degradation of pesticides; microbial degradation of pesticide combinations (Kaufman et al., 1970; Kaufman, 1977); and soil enrichment and subsequent isolation of effective microorganisms. The relationship of data obtained in a perfusion system to actual environmental persistence is tenuous at best.

An interesting observation associated with the isolation of effective microorganisms from perfused soils is that bacteria appear to be the predominate organisms isolated from soils enriched by this mechanism. In contrast, soil fungi appear to be the organisms predominately isolated from soils enriched by simple shake culture techniques.

Soil Biometry

Several biometer type systems have been used to study degradation and metabolism of ^{14}C-pesticides in soils. Two of these methods have been used extensively in our laboratory. Although two of the systems can be used to

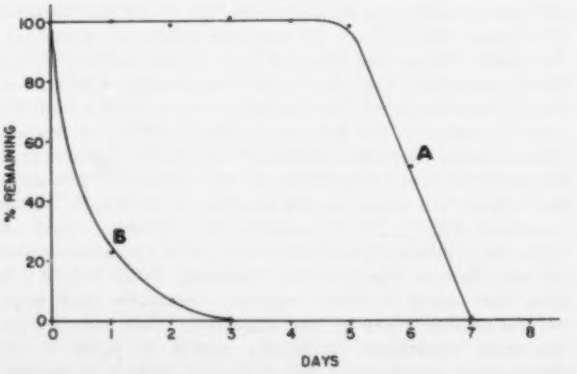

Figure 29. Microbial degradation of 2,2-dichloropropionic acid (dalapon) in (A) a previously untreated soil perfused with an aqueous solution of dalapon; and (B) the same soil re-perfused with a second aqueous solution of dalapon (Kaufman, 1966).

study pesticide metabolism by isolated organisms, they are more commonly used with the total microbial population in soil considered as a tissue or unit organism. One system involves the "soil biometer" flask developed by Bartha and Pramer (1965) (Figure 30). When ^{14}C-labeled pesticides are incorporated into the soil, the apparatus and method of analysis enable both $^{14}CO_2$ and total CO_2 production to be monitored at frequent intervals for prolonged periods, and all measurements are made on the same soil sample without significant exposure to atmospheric CO_2. Although it was suggested by the developers that the magnitude of the difference in amount of CO_2 produced by treated and untreated soil can be used as a measure of the extent to which an unlabeled pesticide molecule was oxidized, this approach would be inaccurate with pesticides which stimulate microbial activity in addition to serving as a substrate. If this latter approach is used it should be mandatory to have product dissipation data obtained by soil extraction, which basically supports the dissipation rate indicated by total CO_2 evolution. Use of ^{14}C-labeled pesticides provides a far more simple and accurate indication of actual pesticide metabolism in soil.

The soil biometer flask has been used successfully to study microbial metabolism of numerous pesticides in soil. Several limitations are apparent, however. One limitation of this technique is encountered when volatile pesticides which can be trapped in base (KOH) are examined in this system. It then becomes essential to determine by organic solvent extraction or precipitation of evolved CO_2, how much of the ^{14}C material trapped in the base is actually $^{14}CO_2$ or volatile ^{14}C-pesticide residues. This problem is avoided in the system developed by Anderson (1975) or the flow-through system (Kearney and Kontson, 1976) which will be discussed later.

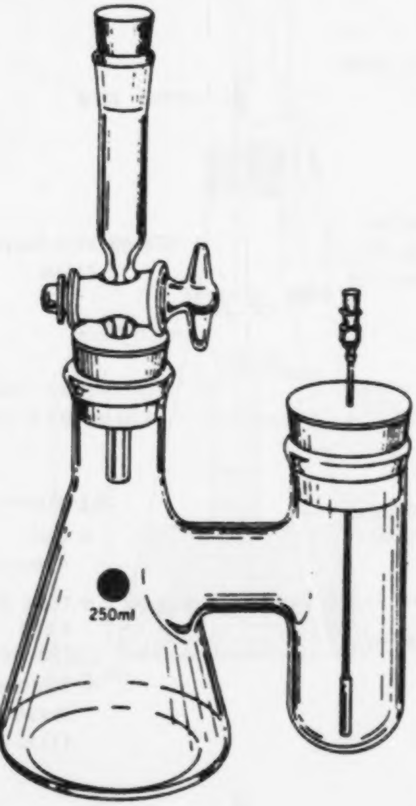

Figure 30. Soil biometer flask (Bartha and Pramer, 1965).

A second concern or limitation for the biometer flask involves the aerobicity of the system during long-term incubation experiments. The soil microbial population consumes oxygen and evolves CO_2. The CO_2 is removed by the trapping solution in the sidearm of the biometer flask. The concern thus becomes whether or not the atmosphere of the flask ultimately goes anaerobic with an ever increasing content of N_2. Although the author has not seen any data which clearly supports this phenomenon there is indirect evidence to support it. In some incubation experiments with biometers a negative pressure within the flask is observed when the stopper is removed from the needle and bubbles of air emerge from the needle point into the trapping solution. A second indication is that in comparative long term experiments we have observed that pesticide degradation to $^{14}CO_2$ is much more extensive in a flow-through system than in the biometer flask.[2]

Several alternatives have been used to circumvent this phenomenon. In some investigations where total CO_2 evolution is not a concern, the investigators have simply left the syringe needle open so that as a negative pressure develops within the flask, additional air is drawn in to replace that used. While this in effect partially solves the problem, it still allows for an increasing N_2 atmosphere which would be out of balance with a more normal aerobic environment. A second alternative used by at least one industry has been to simply attach the open end of the syringe needle to a low pressure O_2 manifold. Thus, as the O_2 is utilized within the flask it is replaced by O_2 drawn in by the negative pressure. With these limitations in mind, however, the biometer flask has proven to be one of the most useful methods of studying pesticide metabolism in soil in the laboratory.

Several alternatives to the Bartha-Pramer biometer flask have been developed. One, originally developed by Anderson (1975), and subsequently modified slightly by Marvel et al. (1976) is shown in Figure 31. The advantage of this system over the Bartha-Pramer biometer flask is obvious in that it allows free exchange of air with the outside atmosphere, in addition to providing a method of trapping volatilized pesticide or degradation products. This system also is readily adaptable to use with pure culture metabolism in aqueous media. Its major limitation is the time involved in processing the granular CO_2 trapping material. This must be removed periodically, solubilized, and the $^{14}CO_2$ retrapped prior to scintillation counting. The modifications made by Marvel et al. (1976) allow for separate analysis of evolved $^{14}CO_2$ and atmospheric CO_2.

Another system was developed by Loos, Kontson, and Kearney (1977) in our laboratory (Figure 32). The system involves a free-drainage mixture of soil, sand and organic substrate over a layer of pea gravel which permits watering to water-holding capacity and recirculation of drainage water for easy maintenance of a constant moisture content. The flask is continually open to air, with Ascarite and KOH traps to remove CO_2 from incoming and outgoing air, respectively. Evolved $^{14}CO_2$ is absorbed in the KOH trap, which can be removed periodically for analysis by liquid scintillation counting. Use of a replaceable scintillation vial for this trap permits easy and rapid sampling. The authors examined the degradation of selected ^{14}C-labeled herbicides (TCA [trichloroacetate]; 2,4-D[(2,4-dichlorophenoxy)acetic

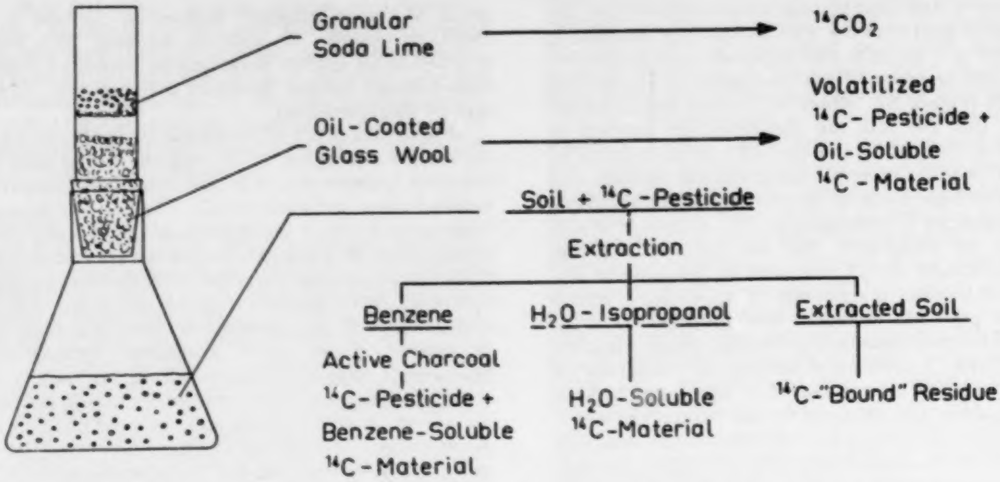
Granular Soda Lime
$^{14}CO_2$
Volatilized ^{14}C- Pesticide + Oil-Soluble ^{14}C - Material
Oil-Coated Glass Wool
Soil + ^{14}C -Pesticide
Extraction
Benzene
Active Charcoal
^{14}C- Pesticide + Benzene-Soluble ^{14}C - Material
H_2O - Isopropanol
H_2O-Soluble ^{14}C-Material
Extracted Soil
^{14}C-"Bound" Residue

Figure 31. Culture flask system for investigation of degradation, volatilization and binding of ^{14}C-labeled pesticides in soil or culture solutions (Anderson, 1975).

acid]; chloroprophem[isopropyl m-chlorocarbanilate) and nonpesticide chemicals (quanine, cytosine, and urea), and found them to follow expected degradation patterns, i.e., either immediate rapid degradation, or a lag period followed by an increased rate of degradation. Actually, the degradation patterns observed with this system appeared to be similar or intermediate to what one may expect to see in either the soil perfusion system or a soil biometer flask. A limitation of the system was observed, however, when the authors attempted to monitor the degradation of subsequent additions of the chemicals examined. Unexpected degradation patterns, i.e., no rapid degradation with elimination of the lag period, were observed with the second additions of labeled substrates. This led the authors to conclude that the substrates must be mixed with the soil and sand before establishment of the soil flasks.

This system has actually only been used in a limited number of investigations and does not appear to have been developed to its full potential. Considerable time, effort, and detail is involved in setting up this system. It also lacks any mechanism for readily distinguishing between volatilized products and evolved $^{14}CO_2$. Nonetheless, it would appear to have some interesting potential for removal and examining the production of more polar water soluble products produced during pesticide metabolism in soil and removed in the leachate.

More advanced and seemingly complex systems have been developed and are being utilized by other laboratories. One such system which the author has observed involves a system similar to that used by Lichtenstein, Fuhremann, and Schulz (1974). This system involves both an enclosed growing crop and soil. A partition through the middle of the system separates the plant tops from the soil and roots and permits $^{14}CO_2$ and volatilized products from each half of the chamber to be monitored separately. Soil degradation patterns observed in these systems are those which might be expected. Generally, soil degradation in the presence of a growing plant occurs much more rapidly, presumably due to the rhizosphere effect on the soil microbial population.

Flow-Through Systems

A wide variety of flow-through systems have been used in soil pesticide metabolism investigations. They have

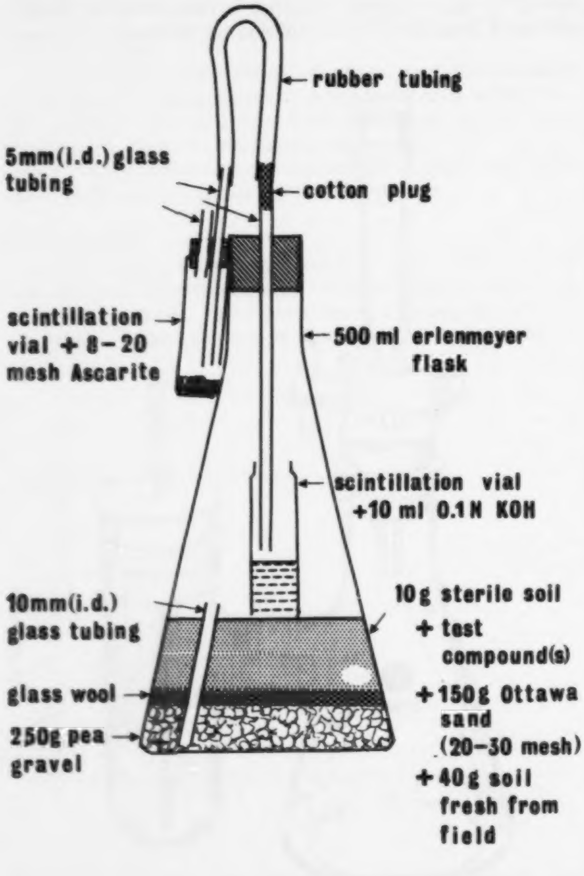
rubber tubing
5mm(i.d.) glass tubing
cotton plug
scintillation vial + 8-20 mesh Ascarite
500 ml erlenmeyer flask
scintillation vial +10 ml 0.1 N KOH
10mm(i.d.) glass tubing
10 g sterile soil + test compound(s) + 150 g Ottawa sand (20-30 mesh) + 40 g soil fresh from field
glass wool
250 g pea gravel

Figure 32. Soil and sand incubation system developed by Loos et al., (1977).

varied anywhere from a few flasks connected in series to the complex manifold assemblies illustrated in Figure 33. While they do offer several advantages such as: (a) the conduct of soil respiration experiments under more precisely defined conditions; (b) the use of different gaseous environments; and (c) can be operated on either positive or negative pressure but they are complex in their design and generally require a greater expenditure of funds for the necessary equipment. Practical solutions to the problem of pesticide persistence in soils may well depend upon laboratory

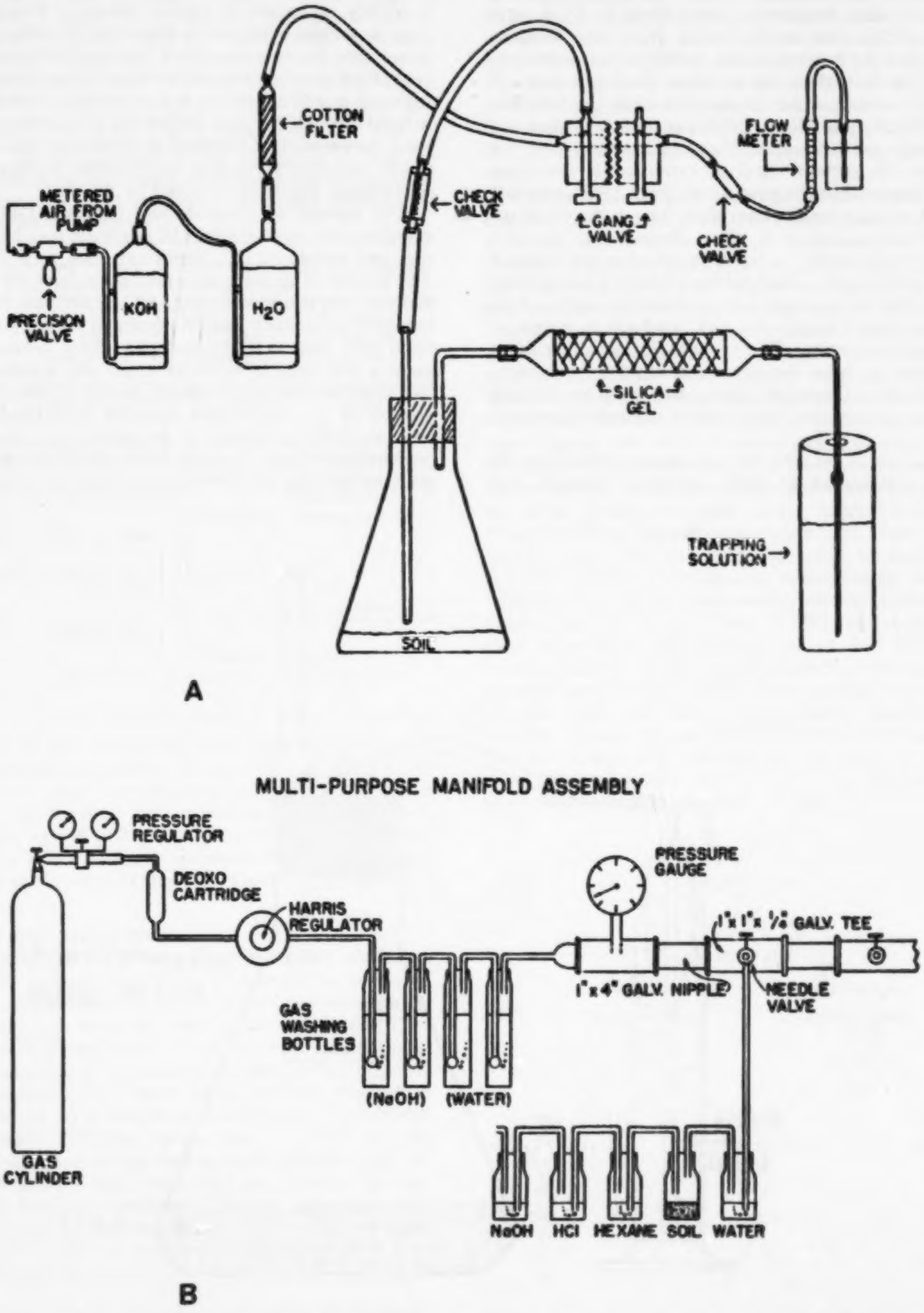
COTTON FILTER
METERED AIR FROM PUMP
CHECK VALVE
GANG VALVE
FLOW METER
CHECK VALVE
PRECISION VALVE
KOH
H_2O
SILICA GEL
TRAPPING SOLUTION
SOIL
A
MULTI-PURPOSE MANIFOLD ASSEMBLY
PRESSURE REGULATOR
DEOXO CARTRIDGE
HARRIS REGULATOR
PRESSURE GAUGE
1"x 1"x 1/4" GALV. TEE
1"x 4" GALV. NIPPLE
NEEDLE VALVE
GAS WASHING BOTTLES
(NaOH) (WATER)
GAS CYLINDER
NaOH HCl HEXANE SOIL WATER
B

Figure 33. Flow-through incubation systems developed by (A) Goswami and Koch (1976), and (B) Parr and Smith (1969).

investigations conducted under precisely defined and reproducible conditions. A number of different types of manifolds have been employed with the constant aeration method in microbial respiration studies in recent years (Stotzky, 1965). Means of controlling the flow rate in these systems have included the use of screw clamps (Waksman and Starkey, 1924), standard sections of capillary tubing (Bartholomew and Broadbent, 1950; Stotzky, Ryan, and Mortensen, 1958), and needle valves (Parr and Reuszer, 1962). A review by Parr and Smith (1969) of past investigations revealed that often the aeration flow rate was not measured. However, some researchers have used bubble counting (Stotzky and Goos, 1965), gas meters (Potter and Snyder, 1916), and flowmeters (Parr and Reuszer, 1962) for this purpose. In certain types of experiments involving desorption phenomena (Papendick and Parr, 1965), accurate control and measurement of aeration flow rates are of utmost importance, and it is often desirable to conduct preliminary experiments to establish whether the aeration flow rate is limiting. In a study of the effect of aeration flow rate of CO_2 free air on organic matter decomposition, Parr and Reuszer (1962) found that CO_2 production increased markedly with increasing flow rate. As indicated earlier in this discussion we have also seen more rapid and extensive degradation of ^{14}C-labeled pesticides in flow-through systems than in biometer flasks used in comparable experiments.

An advantage of the flow-through system is that they do permit characterization of both volatilized products and $^{14}CO_2$ evolved. Volatilization losses of the trapping solvent can be problematic when a volatile organic solvent such as hexane is used. This problem is reduced somewhat by the use of ethylene glycol, ethanolamine or similar type traps. A solid compound such as silica gel has been used in some investigations. The recent use of polyurethane plugs (Kearney and Kontson, 1976) provides a simple trap which is readily replacable at regular intervals. These plugs can then be soxhlet extracted to determine volatilized products. In our own laboratory we have been successful in extracting volatilized products trapped on these plugs by simply placing them in a 50 cc syringe and pumping a known volume of solvent through the plug. Before the polyurethane plugs are used, however, it is essential to determine that any potentially volatile materials anticipated during pesticide degradation are indeed trapped by the plug.

The system of Kearney and Kontson (1976) permits simultaneous measurement of pesticide loss by volatilization and metabolic CO_2 evolution from soils (Figure 34). The system is maintained with an air flow which flushes evolved CO_2 and volatilized pesticide residues from the incubation flask. Each flask is equipped with a polyurethane foam plug held in a sintered glass filter funnel. This apparatus was used to examine vapor and metabolic loss of two dinitroaniline herbicides from soil (Table 11). A comparison of the KOH traps revealed no difference in the amount of $^{14}CO_2$ trapped in the presence or absence of the polyurethane plugs. Analysis of the plugs divided into four sections showed that 97% of the butralin volatilized was

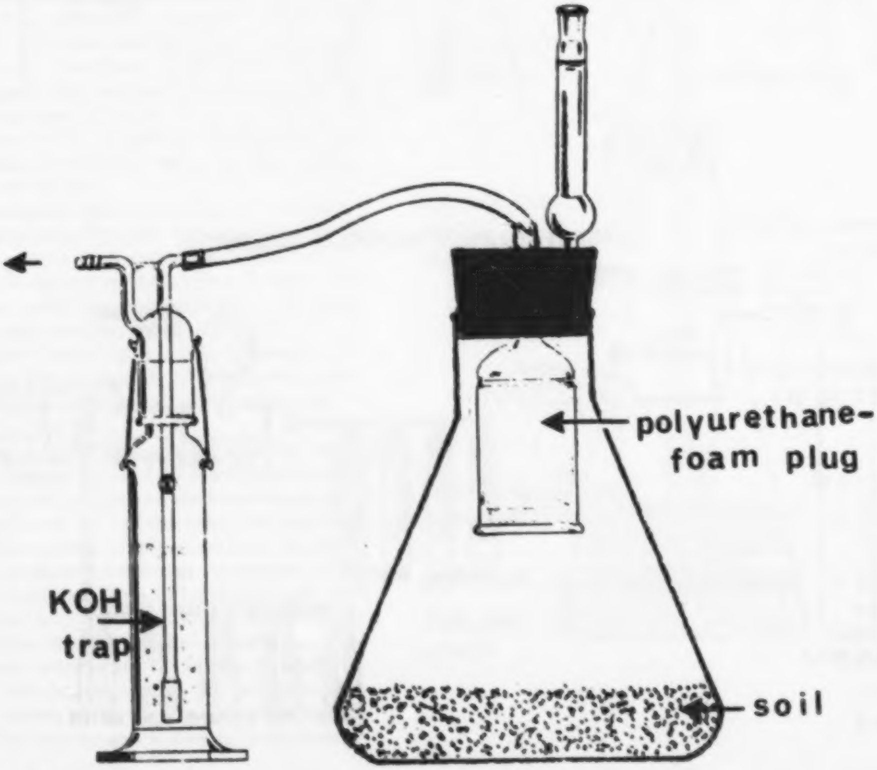

Figure 34. Flow-through system developed by Kearney and Kontson (1976).

Table 11—Vapor and Metabolic Loss of Butralin and Trifluralin from Matapeake Silt Loam (10).[a]

	Trapped ^{14}C as % of total applied					
	Butralin			Trifluralin		
Week	Plugs	$^{14}CO_2$	Total	Plugs	$^{14}CO_2$	Total
1	0.45 (82)	0.10 (18)	0.55	4.42 (95)	0.18 (5)	4.60
2	0.43 (84)	0.08 (16)	0.51	0.86 (81)	0.20 (19)	1.06
3	0.37 (72)	0.15 (28)	0.52	1.16 (69)	0.49 (31)	1.65
Total	1.25	0.33	1.58	6.44	0.87	7.31

[a] Since there was no difference in the amount of ^{14}C lost as volatile products in the light or dark, the values presented represent averages of four replications. Numbers in parentheses indicate percent ^{14}C appearing in plugs and CO_2.

Table 12—General Sequence of Events Occurring in a Waterlogged Soil as Related to Redox Potentials

Event	Eh_7 (millivolts)	Nature of Microbial Metabolism	Formation of Organic acids
Disappearance of O_2	+600 to +350	Aerobes	None
Disappearance of NO_3	+500 to +200	Faculative Anaerobes	Some accumulation after addition of organic matter
Formation of Mn^{2+}	+400 to +200		
Formation of Fe^{2+}	+300 to + 150		
Formation of S^{2-}	0 to −150		Rapid accumulation
Formation of H_2	−150 to −220	Obligate anaerobes	Rapid decrease
Formation of CH_4	−150 to −220		

recovered in the first centimeter of the plug and 1% of each of the remaining three 1-cm sections. Extraction of the KOH solutions with ethyl acetate revealed that no butralin escaped through the plug. Results obtained with this system indicated that loss of trifluralin by volatility from soil surfaces was the major loss mechanism during the first three weeks after application (Table 12) (Kearney and Kontson, 1976).

Anaerobic Soil Metabolism

The investigation of pesticide metabolism in anaerobic soils has only recently become a requirement for pesticide registration. Consequently, only limited information is available for comparative methodological assessment. Two principal methods have been utilized to attain anaerobicity: (1) The atmosphere of an incubation chamber is purged of oxygen by substitution of inert gases carefully purified of their oxygen; or (2) a sample is water-logged to decrease rate of oxygen diffusion below that required by the microbial population within that sample. Because some inert gases are toxic to some microbial populations, the second method has been preferred. To date, anaerobic soil metabolism experiments have been conducted in the same types of flow-through or biometer systems discussed previously, but with the above considerations for aeration in mind.

The recommended method for establishing anaerobicity is flooding (EPA, 1975), with sequential evacuation and replacement of the atmosphere with nitrogen. Flooding or waterlogging a well-aerated soil can have an effect on many oxidation-reduction systems, which, in turn, may indirectly affect pesticide metabolism, fate and environmental behavior. The tendency in recent anaerobic investigations has been simply to use the same soil types used in aerobic investigations. While this has specific merits it may not actually yield data which indicates what actually happens to a pesticide in a truly anaerobic environment. Soils which have a low level of aerobic microbial activity may only very slowly, or perhaps never, become fully anaerobic. Thus, pesticide degradation studies conducted in such soils may not actually yield meaningful data as to potentially significant anaerobic pesticide metabolites or degradation pathways.

Recent reports show that certain pesticides appear to degrade more rapidly in soils under biologically active anerobic conditions compared with aerobic conditions (Guenzi and Beard, 1968; Hill and McCarty, 1967; Kallman and Andrews, 1963; Parr, Willis, and Smith, 1970; Raghu and MacRae, 1966; Willis, Wander, and Southwick, 1974). Degradation pathways and products produced under anaerobic conditions may differ from those observed under aerobic conditions. For example, separate aerobic and anaerobic pathways have been reported for trifluralin (α, α, α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) degradation (Figure 35) with anaerobiosis resulting in faster degradation (Probst et al., 1967). The aerobic pathway involves loss of the propyl groups followed by reduction of the nitro groups, whereas under anaerobic conditions, reduction of the nitro groups occurs first. These and other results imply that soil flooding could be imposed as a cultural practice for inducing anaerobiosis, thereby accelerating the rate of pesticide degradation in contaminated fields. Parr, Willis, and Smith (1970), however, observed that anaerobic degradation rates for DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane] varied with the method used to create anaerobiosis, and that degradation in flooded soils was only slightly faster than in aerobic systems. Thus it appears that factors other than mere exclusion of oxygen (e.g., possibly the intensity of reduction) may determine the rate of pesticide degradation in anaerobic environments.

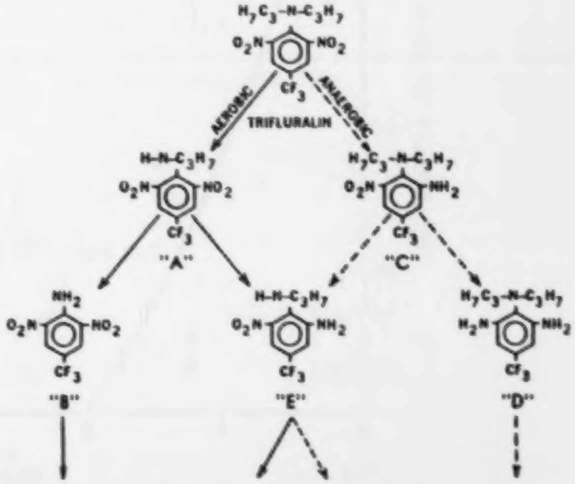

Figure 35. Postulated pathway of trifluralin degradation in soil (Probst et al., 1967).

The sequence of chemical transformations resulting from microbial metabolism in waterlogged soils has been well documented (Table 12) (Parr, 1969; Redman and Patrick, 1965; Takai and Kamura, 1966; Turner and Patrick, 1968). One method of characterizing the intensity of reduction in anaerobic systems is by the measurement of redox potentials (Eh). The usefulness and limitations of measuring redox potentials in natural systems have been discussed by Bohn (1968, 1969, 1971). Although little is known concerning the direct effect of soil redox potential on the degradation rate of specific pesticides, there does appear to be some relationship between pesticide degradation and redox reactions (Ecobichon and Saschenbrecker, 1967; Glass, 1972; Guenzi, Beard, and Viets, 1971; Wedemeyer, 1966).

The research of Willis, Wander, and Southwick (1974), and Southwick, Willis, and Keszthelyi (1977) indicate that degradation of trifluralin in anerobic soil is closely related to the redox potential. They used a system for controlling redox potential in soil suspensions to investigate the relationship between oxidation-reduction potential and the rate of trifluralin degradation. Redox potentials of +450, +250, +150, +50, 0, and -50 mV were imposed on trifluralin amended soil suspensions for 21 days. Results indicated that exclusion of oxygen by soil flooding initiated rapid trifluralin degradation only when the Eh decreased below a critical range between +150 and +50 mV (Figure 36). There was no clearcut relationship between trifluralin degradation and microbial respiratory activity as determined by CO_2 evolution. Subsequent investigations in sterile and nonsterile systems indicate that the initial nitro group reduction occurs more rapidly by chemical means, and that the second reduction is microbiological. These studies indicate the significance of controlling the redox potential in anaerobic soil degradation of pesticides. Thus, it would seem important to consider doing anaerobic pesticide investigations at specific soil redox potentials, or making pesticide additions to anaerobic soil only after specific redox potentials are established, or both.

Chemical vs Microbial Degradation

Soil microbial populations, chemical reactions, and physical processes are influenced by soil organic matter, moisture, temperature, pH, cation exchange capacity, aeration, and numerous other parameters. Soil chemical reactions and physical processes affecting pesticide behavior frequently respond in ways simulating microbial population responses. Thus, careful, diligent investigation is necessary to distinguish accurately between, chemical, physical, and biological processes affecting the environmental fate and behavior of pesticides in soil.

Occasionally we have encountered chemicals whose degradation pattern in soil simulates a microbiological phenomenon, when in actual fact, the degradation process is either totally chemical, or dependent upon an initial chemical reaction followed by microbial metabolism. Generally, these reactions can be sorted out by using various types or combinations of soil sterilization pro-

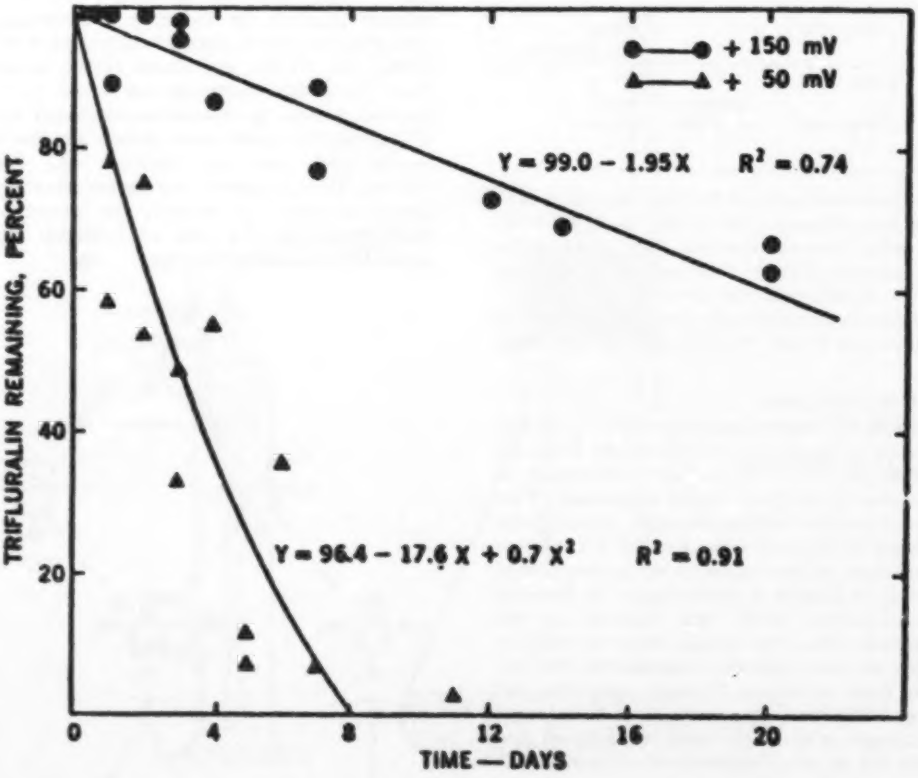

Figure 36. Disappearance of trifluralin from soil suspensions as a function of redox potential and time (Willis, Wander, and Southwick, 1974).

cedures and investigating various types of purely chemical systems suspected of affecting the pesticide. Chemicals which have fallen into this category are: amitrole (3-amino-s-triazole); MH (1,2-dihydro-3,6-pyridazinedione); and ETU (ethylenethiourea) (Figure 37).

Investigations in our laboratory demonstrated that amitrole degradation was due to chemical processes in soil (Kaufman et al., 1968) whereas MH and ETU degradation was initially chemical followed by microbial activity (Kaufman and Fletcher, 1973; Kaufman and Kalayanova, 1976). A comparative study of soil sterilization methods revealed that amitrole degradation occurred in both KN_3-treated and ethylene oxide sterilized soils, but not in autoclaved soil (Figure 38). Although KN_3 is not a sterilant per se, it was essentially complete in its inhibition of microbial activity at the concentrations used in this investigation. The degradation of amitrole in a sterile environment indicates that soil microorganisms at least are not directly involved in amitrole degradation. Subsequent investigations (Kaufman et al., 1968; Plimmer et al., 1967) indicated that amitrole is highly susceptible to degradation by free radical systems. Soil sterilization by any mechanism drastically alters the reactivity of the soil. In this investigation, soil sterilization by autoclaving ultimately destroyed the free radical generating mechanism, as well as the soil's biological properties.

In similar investigations (Kaufman and Fletcher, 1973; Kaufman and Kalayanova, 1976) it was demonstrated that ETU and MH are initially degraded by chemical mechanisms, but further degradation was microbiological. In chemically sterilized soil MH degraded initially to maleimide and maleamic acid, whereas ETU was degraded to ethyleneurea. In both instances, an active microbial population was essential for further degradation to occur in soil. Both parent chemicals were also susceptible to degradation in free radical generating systems.

Other chemical degradation reactions may also occur in soil. Chemical transformations in soil, mediated through water functioning as a reaction medium, a reactant, or both are widespread phenomena. The chemical reactions that occur in soil may be catalyzed by clay surfaces, metal oxides, metal ions, organic surfaces, and organic materials that can be separated from the soil. Additionally, extracellular enzymes which are widely distributed and stabilized in soil, play a significant role in the degradation of many pesticides and represent the transition point between chemical and intracellular microbiological breakdown. As indicated earlier in this section, considerable care is necessary to distinguish accurately between chemical, physical and biological processes in soil. For purposes of pesticide registration, however, it would seem most expeditious to delineate the pathway(s) and rate of dissipation from soil and to identify or characterize the intermediate products involved insofar as reasonably possible. The exact mechanism (microbial vs chemical) whereby a given pesticide degrades could be considered of more academic than practical significance.

Soil Sterilization

Comparisons of pesticide persistence in autoclaved and nonsterile soils have frequently been the sole criteria for concluding microbial transformation as the mechanism of pesticide dissipation. Such conclusions are speculative and merely contribute to the quandary already surrounding the environmental fate and behavior of pesticides. Autoclaving so disrupts the soil chemical and physical structure as to confound most other mechanisms of dissipation which might respond in a similar manner to environmental factors generally known to affect microbial populations. As illustrated in Figure 38, discussed in the preceding section,

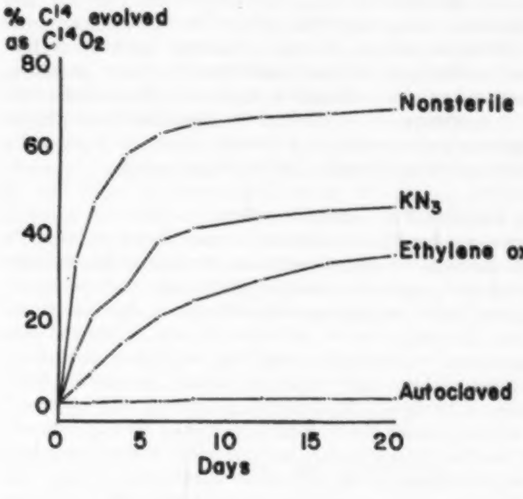

Figure 38. Effect of soil sterilization method on amitrole degradation in soil (Kaufman et al., 1968).

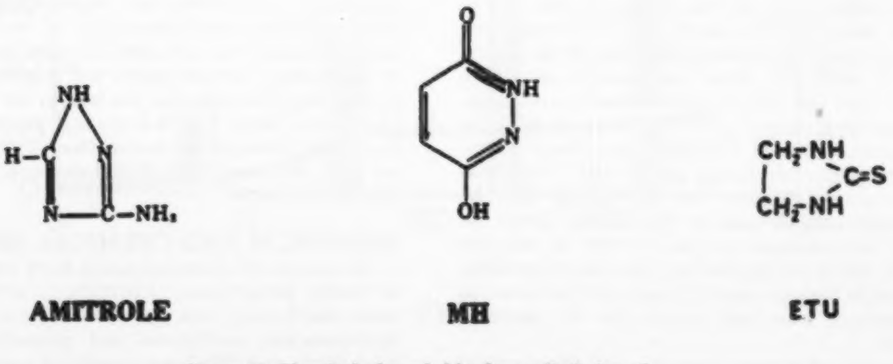

Figure 37. Chemicals degraded by free radicals in soil.

autoclaving destroyed or eliminated the chemical mechanisms of amitrole degradation in soil.

Additional methods of eliminating, controlling, or reducing microbiological activity should be employed to assess the contribution of the soil microbial population. Methods such as fumigation, gamma irradiation, specific microbial inhibitors, or dry heat should be used to augment the more traditional methods. Whatever technique(s) are employed, however, the results must necessarily be interpreted in light of the known effects of that method on other chemical and physical processes. We recently examined the effect of soil sterilization methods on 3-chloroaniline behavior in nonsterile soil (Fletcher and Kaufman, 1974) and soil sterilized by KN_3, $HgCl_2$, ethylene oxide, and autoclaving. Treatments were monitored for ^{14}C volatilization, the formation of organo-soluble products and bound residues. Highest levels of ^{14}C volatilization occurred from autoclaved and KN_3 treated soils, whereas lower ^{14}C-volatilization occurred from nonsterile, $HgCl_2$, and ethyleneoxide treated soils. Significant differences were observed in the distribution of ^{14}C activity in organosoluble fractions and soil bound residues among the various treatments. Differences in the distribution of ^{14}C-activity in the humic and fulvic acid, and humin fractions of the soil bound residue were also apparent. Thus, the significance of any single soil sterilization method must be interpreted within the limitations of its own individual effects on soil processes. Because of the unreliable effects of soil sterilization methods on pesticide behavior and product formation in soil, and their negligible value in assessing the microbial involvement in pesticide metabolism, we consider that "sterile controls" have only limited or no value in soil dissipation protocols.

Other Factors For Consideration

Additional factors which must be considered in conducting soil metabolism experiments in the laboratory include: chemical and physical properties of the pesticide being investigated; soil absorption characteristics, formulations, available labeling patterns, anticipated costs, mode and rate of application, replications, sampling frequency, analytical procedures, soil type and moisture content, incubation temperature, anticipated degradation pathways and mechanisms, and, of course, the overall objective of the experiment. Under certain circumstances any one or several of these factors may determine what sort of an incubation system will be utilized in a soil metabolism investigation. For example, if the vapor pressure of a given chemical or anticipated degradation product is sufficient to expect that volatilization from the soil may be a problem, then a method which permits characterization of volatile losses should ill be used.

Most pesticides are of relatively low water solubility and are thus most easily added to soil in limited amounts of organic solvent which is subsequently allowed to evaporate. An awareness of the volatilization properties of the chemical is critical at this point. Also, consideration should be given to the amount and type of solvent used to avoid supplying extraneous carbon sources, or possible toxicity to the microbial population present. Generally, we attempt to apply insoluble pesticides in minimal amounts (0.1 - 0.2 ml/50 g soil) of acetone or ethanol. The soil should then be thoroughly mixed and watered to the desired level; the recommended soil moisture content is 75% of 0.33 bar moisture. (EPA, 1975). Water soluble pesticides are preferably applied in sufficient aqueous solution to bring the soil to its desired moisture level and incorporate the pesticide simultaneously.

Unless a formulated chemical is being used, the chemical should be of known purity, i.e., technical or reagent grade. Radioactive labeling should be in a position of the molecule which allows characterization of significant breakdown products.

A minimum of 2 or 3 replications are used. Sufficient units are established to permit periodic sacrificing and characterization of degradation rates and product appearance and disappearance. Maximum incubation periods are no more than 1 year since incubations longer than a single growing season probably become misleading due to the equilibrium established in the constant incubation conditions of a laboratory study. A single temperature of 25°C is used for metabolism studies. A minimum of two temperatures are used for degradation rate studies.

Incubated soils are exhaustively extracted by a sequence of solvents. Usually, this involves the least nonpolar solvent in which the parent product has a high degree of solubility followed by methanol. While the EPA Registration Guidelines (1975) have generally indicated that "product identification should be conducted on all materials comprising more than 10% of the initial application, or 0.1 ppm, whichever is greater," our policy has been to identify, or at least characterize, any products which can be isolated in sufficient quantity and purity to permit accurate identification by mass spectrometry, in addition to GC and NMR characterization. Characterization of all products may include TLC mobility, GC retention time, relative polarity, UV absorbance on TLC, radioactive content (% of original or total), or possible TLC spray reactions.

While many of our earlier investigations were conducted in a single soil type (Hagerstown silty clay loam) we generally attempt to conduct investigations in a minimum of 2 different soil types. These usually include a light sandy soil and a moderately heavy loam. Before using the soil it is dried to a workable moisture content and screened through a 2mm sieve before weighing into incubation flasks. It is important to stress that all possible care should be taken to avoid letting the soil air dry and damage the soil population. Typically, we sieve large volumes of soil (40-80 lb) and store it at 5°C in a plastic bag or ceramic or stainless steel vessel. Plastic bags are useful in that they hold the moisture content stable and permit the soil to "breathe." However butylphthalate products can contaminate the soil and present subsequent analytical problems.

After preparation the soil is weighed into the incubation flasks. Care is taken to maintain an adequate ratio of head space versus soil volume. Fifty g of soil (dry wt) is usually adequate for a 250 ml biometer type flask. The moisture content is predetermined so that samples can be adjusted to correct moisture level after treatment.

The ultimate objective of the experiment, of course, determines which of the various preceding factors are of utmost importance and which type of incubation system is used. If the objective is to simply assess the biodegradability of the chemical and/or isolate soil microorganisms capable of degrading the chemical, we tend to use the perfusion approach if suitably simple analytical procedures are available. Other more detailed metabolism experiments, however, are best conducted with ^{14}C-labeled compounds in one of the other systems.

BIOLOGICAL AND CHEMICAL ASSAYS

Biological and chemical assays have been primarily used to study persistence, adsorption, and movement of pesticides through soil, and the effects of various soil and environmental conditions and properties on pesticide behavior in soil. We have frequently used these assays in conjunction with other soil metabolism experiments as a further indication of a loss of toxicity as affected by whatever experimental parameters are being investigated. For example, bioassays of sterile and inoculated microbial culture solutions added to soil after varying periods of incubation have been used in investigations of s-triazine herbicide degradation (Figure 39), (Kaufman, Kearney, and Sheets, 1965; Kaufman and Blake, 1970). Similarly, they have been used to compare the loss of phytotoxicity from soil with loss of $^{14}CO_2$ from ^{14}C-EPTC treated soil (Figure 40). (Kaufman, 1967). The difference between the slow release of $^{14}CO_2$ from labeled EPTC and the more rapid loss of phytotoxicity, as revealed by bioassay, indicates detoxication without appreciable mineralization of the ^{14}C-containing moiety of the molecule.

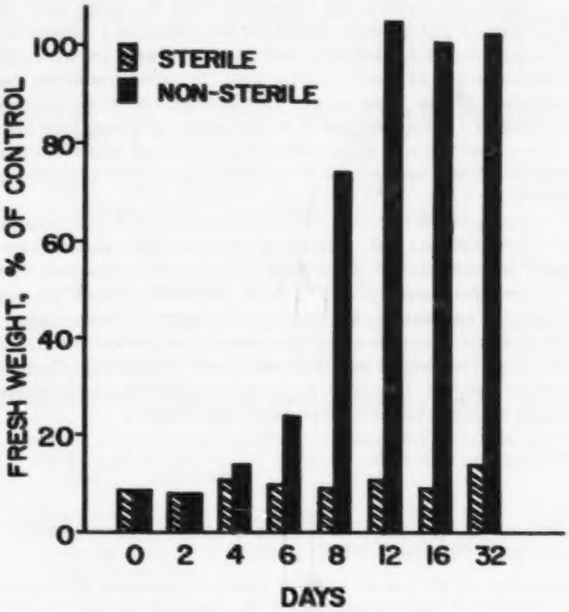

Figure 39. Oat seedling soil bioassay of A. fumigatus culture solutions containing simazine (Kaufman, Kearney, and Sheets, 1965).

Bioassays quantitatively measure the biologically active concentration of known toxicants. Bioassays involve the measurement of a biological response by living plant, microbe, or animal organisms in order to determine the presence and/or concentration of chemical in a substrate. A soil bioassay involves the growing of an indicator organism in a pesticide treated soil, and then comparing the response of the test organism to similar organisms cultured in untreated soil or in soil containing known pesticide concentrations. Two basic assumptions are made when using bioassays: (a) that the organisms show increased injury in an order related to the pesticide concentration; and (b) that the responses obtained are reproducible.

Bioassays and chemical-physical assays each contribute information of value and each are useful for certain circumstances. A major advantage of the bioassay is the assurance that the toxic activity of the pesticide molecule is what is being measured. It is not usually necessary to extract the pesticide from the substrate, thus circumventing the problems involved with the extraction procedures that are often needed for chemical assays. Bioassay procedures are usually more economical, less difficult to perform, and do not require as much expensive equipment as chemical and physical analytical methods. However, some difficulties exist and must be considered when developing or using a bioassay. Growth is directly influenced by ambient environmental conditions such as light, temperature, watering method and level, and humidity. Variations in these factors may cause a greater biological response than the pesticide under test. Thus, the environmental and edaphic factors

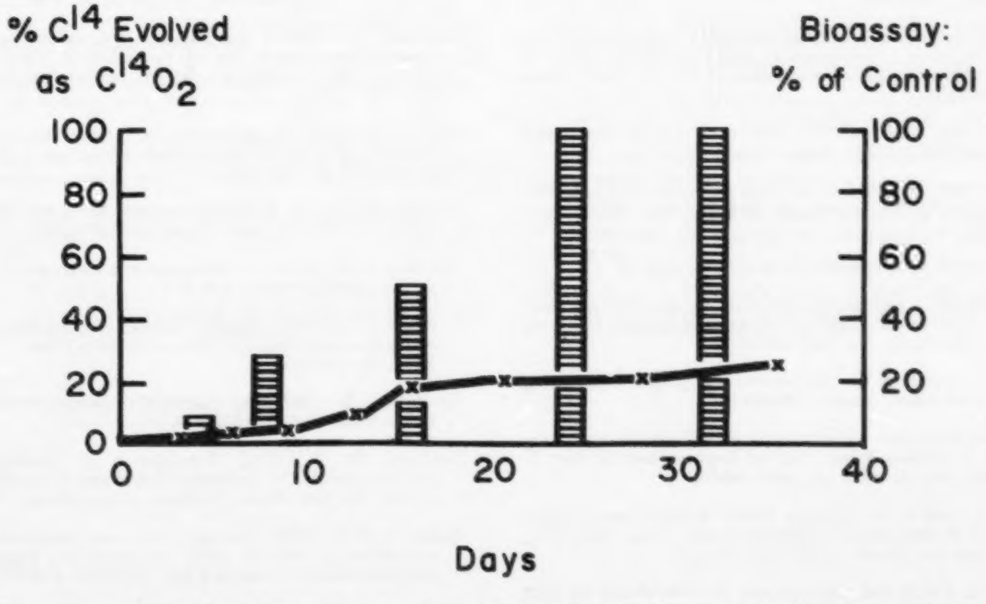

Figure 40. Persistence and degradation of EPTC in soil as determined by oat seedling bioassay and $^{14}CO_2$ evolution (Kaufman, 1967).

must be very closely controlled. Untreated organisms and known standards should be included each time an assay is conducted. Species variation in susceptibility to a given pesticide, and in some instances individual organism variation within a species may also be apparent. A comprehensive guide to bioassay, adsorption, absorption, leaching, and microbial interaction methodology has been published (Wilkinson, 1972).

Chemical analyses for pesticides and their degradation products in the environment was for a long time one of the major stumbling blocks in pesticide research. With the development of new instrumentation and procedures it is now possible to determine quantities of pesticides as low as a few parts per billion or trillion. Spectrophotometric and chromatographic instruments have become the major analytical tools in determining pesticides and their degradation products. Although chemical assays are useful in both quantitative and qualitative determinations of pesticide residues, they are generally limited by the ability of the solvents to extract the residues from soil. It is this limitation which has prompted concern for what is referred to as the "bound residue."

Often during soil dissipation experiments, a certain amount of radioactivity remains unextractable and is referred to as "bound residue." A proposed definition of soil bound residues is "that unextractable and chemically unidentifiable pesticide residue remaining in fulvic acid, humic acid, and humin fractions after exhaustive sequential extraction with nonpolar organic and polar solvents." Although the importance of these residues is not fully understood at present, a significant amount of many pesticide residues in soil fall into this category. Thus, it is essential to characterize the distribution of such residues in the soil organic matter matrix.

Stevenson (1965) has suggested that methods used to fractionate soil organic matter can be placed in 2 categories: (1) Those used to fractionate and estimate compounds characteristic of plant tissues; and (2) those based on the division of the humus into subclasses possessing similar solubility characteristics. It is the latter of these two procedures which was suggested as the preferred approach in a recent edition of the EPA Pesticide Registration Guidelines (1975).

The great difficulty with all fractionation procedures, however, is that the methods employed either separate out products which are not definite chemical entities or that fractionation methods may form artifacts which do not have the properties of the original material. Nevertheless, the various fractionation procedures have proved useful for studying soil bound pesticide residues. Additional information is needed, however, to more completely characterize these residues and establish their significance.

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PART 2

Colloquium on Terrestrial Microcosm Technology

Overview of Terrestrial Microcosm Program

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The U.S. Environmental Protection Agency, the U. S. Department of Agriculture, and the Energy Research and Development Administration each are carrying on laboratory model ecosystem investigations of pesticides and other toxic substances. The purposes differ, the exact manners of execution vary, but there are common threads of research that have been brought together in this Colloquium to exposit and discuss. What can we predict about the environmental fate and effects of chemicals based upon data derived from studies in closed, controlled laboratory systems? In the Colloquium on Environmental Chemistry we learned that much concerning the fate of chemicals in the environment is generally predictable from critical measurement of certain properties and activities. In this section we will consider a group of studies that examine effects in integrated systems in relation to the fate of chemicals in microcosms. In looking to the potential future application of these systems vis-a-vis chemical regulation, we will be consciously or sub-consciously evaluating the alternatives and modifications as asymptotic approximations of some idealized "new white rat."

Microcosm research and technology, as presented here, is a direct outgrowth of several trends in environmental and toxicology research over the past decade. As both biological and chemical analytical systems have achieved increased sensitivity and precision in the laboratory and the field, and as our appreciation of the intricacies of biological organization has increased, researchers in the areas of toxicology, ecology, and environmental chemistry have sought increased control of exposures and an increased relevancy of the test to actual field conditions. The outcome of this search has been, predictably, approximately one new system per set of investigators, ranging from soil cores to field plots such as the Zonal Air Pollution System (ZAPS) plots in Montana. Clearly then a re-examination of criteria of performance and of the purposes of these test systems should be part of the colloquy of this Symposium.

In 1973 the U. S. Congress passed PL 93-135 into being, creating the Substitute Chemicals Program. The object of this legislation was to provide evaluation of alternatives to pesticides which might be cancelled, suspended, or otherwise held in suspicion as being "environmentally difficult." Many of the well-established and consequently heavily researched agents were or are in the process of being deregistered or otherwise limited in some way or another. Information about many alternative agents already registered for those uses (or which might be registered for those uses in the near future) was extremely meager. Thus one of the charges to the Office of Research and Development by the Office of Pesticides Programs was to develop test methods which would evaluate the environmental fate and hazard of substitutes for such chemicals as DDT, dieldrin, 2,4,5-T, and methylmercury.

Dr. Norman Glass and Dr. Allan LeFohn at CERL undertook the development of a Laboratory-wide program that consisted of efforts in estuarine/marine systems by Dr. Peter Schoor at Gulf Breeze ERL, freshwater systems by Dr. Dotty Paris at Athens ERL and by Dr. Alan Isensee of USDA/Beltsville under Interagency Agreement with the Duluth ERL, and terrestrial/atmospheric systems by the former National Ecological Research Laboratory at NERC-Corvallis. By 1974 we had started a grants program supporting the extension of the earlier work of Dr. Metcalf on an aquatic-terrestrial ("farm pond") microcosm to a terrestrial monoculture system and initiated an in-house system. At approximately this time Dr. Kearney's group at USDA-ARS, Beltsville began the independent development of the model agroecosystem. We initiated attempts to model mathematically some of the aspects of fate and effects in controlled laboratory systems and in field plots with in-house work by Dr. James Haefner and by a grant to Drs. William Cooper and Erik Goodman at Michigan State University. This latter work is especially closely related to the efforts at the Athens ERL on the Agricultural Run-off Model (ARM) and to experiments in AECOS, the model laboratory stream. In 1976 a grant was given to Dr. Lichtenstein at the University of Wisconsin for studies in their plant-soil-water system, and a contract from the Office of Pesticide Programs was awarded to Mid-West Research Institute to provide basic toxicology data on the test pesticides and Microtine rodents (voles) used in the Metcalf and NERL systems. About that same time Duluth ERL awarded a grant to Dr. Charles Warren of Oregon State University to consider criteria for design of microcosms.

The terrestrial components of this program amount to about $600K/annum in the US Environmental Protection Agency. There have been a number of products: "A Conceptual Model of the Transport of Chemicals in the Environment" (USEPA Ecological Research Series) has been distributed to several thousand people; there have been published, accepted or in submission about 8 research papers; and reports have been delivered at approximately a dozen meetings. The Colloquium on Terrestrial Microcosms reports parts of the USEPA and USDA efforts to date.

It would be appropriate to call attention to some of the concepts in microcosm research. For example, they were referred to earlier as "new white rats." The organismic analogy of an ecosystem, which may be taken to be a collection of interdependent components and processes from which emerge unique properties, appears very apt for microcosms. This analogy has the value of stimulating thinking about the clinical testing methods which might be developed for microcosms and be applicable to assessing the "health" or "illness" of real ecosystems. The analogy has further value in that diverse strains and species have yielded useful information about particular processes, and we should expect the same to be true of microcosms. Thus we would expect that laboratory ecosystems could serve in the development of better tools for environmental hazard evaluation in actual environments, or, to take the opposite approach, that established tools developed to evaluate real ecosystems might be applied in microcosm tests as measures of effect of agents or other stresses to be screened in some manner.

For the resultant data to have meaning in a toxicological sense, we need criteria of operation and evaluation that validly reflect response of components and processes of real ecosystems. This is particularly true in regard to scaling factors. The same questions are not addressed in experiments in which one treats one 150-kg swine, or 3 50-kg dogs, or 750 200-g rats. Each experiment provides a useful fragment of data if the questions were properly posed and correspond to the unique character of the organism involved. The evaluation of the data takes that into account according to criteria developed for that purpose. Similarly, the scale or scope (i.e., complexity) of a microcosm system can best be related to real environments by relevant criteria limiting data evaluation.

The temporal scale of microcosm operations is substantially longer than convenient for the typical acute LD_{50} weeks if not months. On the other hand the systems most likely to be affected and most needing protection from man's chemical activities—forests, agricultural lands, estuaries—exist in that form over centuries or at least decades. We would hope to make ecological decisions with at least the timeliness and precision that we use in chemotherapeutics. It certainly would be evident to have an equivalent of an EEG or glucose tolerance test to identify sensitive environments *re* particular chemicals, as we can epileptics or diabetics. Components of microcosms can respond in short times, but the "grand processes"—nitrogen cycle, carbon cycle, oxygen/water cycle, microelement cycling, succession, selection—follow arrows of time beyond simplistic measurement in 24, 48 or 96 hrs.

In selecting the scale, scope and timing factors of terrestrial microcosm operation, the investigators have made some, perhaps arbitrary decisions that have presuppositions as to the limits on data measurability and validity. Do these assumptions have validity? Can they be tested appropriately? What are the most significant factors limiting the validity of the overall hypothesis tested by a system? One of the express purposes of this Colloquium is to examine the scientific method as applied in these systems and provide constructive criticism as to the ways to make microcosms more accurate, efficient and valid.

Constructed Systems

Predictive Environmental Toxicology of Pesticides in the Air, Soil, Water, and Biota of Terrestrial Model Ecosystems

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ABSTRACT

We summarize ca. 14,000 quantitative and qualitative analyses in our description of the environmental fates of ten insecticides and fungicides within laboratory physical model ecosystems. The intent is the development of a reliable, efficient, and economical method for quickly differentiating toxic substances relative to their varying potentials to induce chronic adverse environmental effects. Since we desire to predict the potential chronic effects of a substance using a brief experimental period and realistic doses, we concentrate primarily on deciphering the fate of the chemical, as opposed to the effects of the chemical. Pesticide toxicity, degradation, accumulation, and distribution are emphasized and characterized. The pesticides that were examined were DDT, methoxychlor, aldrin, dieldrin, fonofos, parathion, methyl parathion, pentachloronitrobenzene, pentachlorophenol, and captan. Their fates and effects were examined in vermiculite, soil, air, water, sediments, leachates, phytoplankton, Zea mays, Limax maximus, Lumbricus terrestris, Armadillidium vulgare, Estigmene acrea, Microtus ochrogaster, Physa sp., Culex pipiens quinquefasciatus, Daphnia magna, *and* Gambusia affinis.

INTRODUCTION

Beneficial selection is the key characteristic of tomorrow's successful pesticide. Those chemicals which cannot control pests without damaging the environment can best be used by the researcher, not the grower. Today's grower is as concerned with keeping his crop and land uncontaminated as he is with keeping it protected. Tomorrow is the very near future. The traditional chlorinated hydrocarbons have all but disappeared from the U.S. market, and the search for the effective but safe pyrethroid is in full swing. Today's pesticide is registered through an agency that must be concerned with the total efficacy of the product, and not just its effectiveness against potential pests. Due to a long tradition of positive achievements, chemical pest control is, and will continue to be, indispensable in any successful pest management program, but the pesticides to be used must be environmentally compatible. Manufacturers, researchers, and regulatory agencies all require reliable and economical methods for predictively screening the environmental effects and fates of candidate pesticides, especially in terrestrial agricultural environments. Our current research is an attempt to help meet this need.

Some of the critical objectives of this study have been to (1) develop a dynamic living interacting physical indoor system in which to study radiolabeled pesticides thoroughly and safely, (2) compare the environmental behaviors of various pesticides under homologous and reproducible agricultural conditions, (3) obtain reliable quantitative and qualitative data economically and quickly, (4) ascertain the comprehensive environmental fates of pesticides coherently in soil, air, water, crops, invertebrate and vertebrate terrestrial animals, and aquatic organisms, (5) work year round regardless of the outdoor weather, (6) produce indoor results that are indicative of the outdoor environmental behaviors of the compounds, (7) determine the effects of different types of soils, (8) test both preemergent and postemergent pesticide applications, (9) assess the short-term environmentally toxic effects of normal doses of pesticides, and (10) predict the chronic behaviors and effects of the compounds investigated.

We often emphasize a "TO DAD" type of evaluation of the toxicant, which is: *TO*xicity, *D*egradation, *A*ccumulation, and *D*istribution. It is important to note that these are interdependent parameters and not independent processes. The above parameters are examined in the following components of the model ecosystem: representative Air, Soil, Plants, Insecta, Crustacea, Annelida, Mollusca, Mammalia, Surface Waters, Sediments, Leachates, Phytoplankton, Zooplankton, Aquatic Mollusca, Aquatic Insects, and Osteichthyes. The entire model ecosystem is maintained for less than 30 days. By definition, one cannot directly determine the *chronic* adverse biological effects of a given compound in a short period of time. However, such a compound's potential chronic hazard can be estimated by comparing its environmental fate (degradation, distribution, etc.), under homologous conditions, with the fates of those compounds which have been studied intensely for long periods of time. Compounds which have similar fates and similar acute toxicities will likely have similar chronic toxicities. To further complete the estimate of the chronic toxicity of the given compounds, the acute toxicities of all of its degradation products should be determined if they have a tendency to accumulate in vital components of the environment; a chemical change in the parent compound does not necessarily bring about detoxification. One of the strongest advantages of using a radiolabeled parent compound is that it enables one to quantitatively keep track of all degradation products even though their chemical identities and toxicities may not yet be known.

Data Presentation and Useful Reference Points

Frequently an outdoor "pesticide residue" as determined by GC is the result of an analysis of either the extractable parent compound only, or a combination of the extractable parent compound plus selected "major" extractable metabolites (derivatives). The results are then commonly adjusted (concentrations increased) based upon an estimate of the extraction efficiency of the parent compound. The quantitative values in the present report were determined by liquid scintillation analyses, not GC, and the entire residue was positively quantified: an individual "total ^{14}C-pesticidal residue" is the combination of "extractable parent derived from the parent compound, and the "unextractable ^{14}C-products" (UN) remaining in the processed sample. The entire extractable fraction (parent plus metabolites) was quantified before it underwent any clean-up procedure and before an aliquot (or all) of it was chromatographically separated into its various components that were identified and quantified. The chemical nature of the unextractable (or bound) radioactive material is unknown since it was quantified by either thermal destructive oxidation or by chemical disintegration; however, it is often assumed to represent conjugated or chelated derivatives of the parent compound or metabolites. All absolute quantities of parent compounds are presented in μg, and the absolute quantities of all ^{14}C-derivatives are presented as μg of parent compound equivalents. All ppm concentrations are μg chemical/g fresh (wet) weight of material. Concentration in air is given as mg/m^3, which is the same as a ppm (w/w) assuming one liter of air weighs one gram.

The initial pesticide concentration in the most massive component of the model ecosystem, the terrestrial substrate, is a useful reference point when comparing the degree of pesticide accumulation in the various components of the model ecosystem. A given system contained either vermiculite or soil (described below) which differ in density, and the simulated acre depth of all systems was ca. 3 inches (7.5 cm). (Outdoor studies frequently assume an acre depth of 6 inches weighing two million pounds.) All individual systems were treated with 5000 μg of a single pesticide (neat), which simulated a realistic application rate of one lb activie ingredient (AI) per acre (or 1.12 kg/ha). For preemergent applications to systems containing soil, this rate resulted in an initial pesticide concentration in substrate of 1.25 ppm; the initial pesticide concentration in vermiculite was 2.08 ppm.

MATERIALS AND METHODS

The basic ecosystem unit (Figure 41) was a 19-liter widemouth glass carboy 46 cm high with a base diameter of 26 cm. The metal lid, which was on the carboy only when air was being trapped, was equipped with inlet and outlet stainless steel tubing to periodically monitor pesticide vapors in

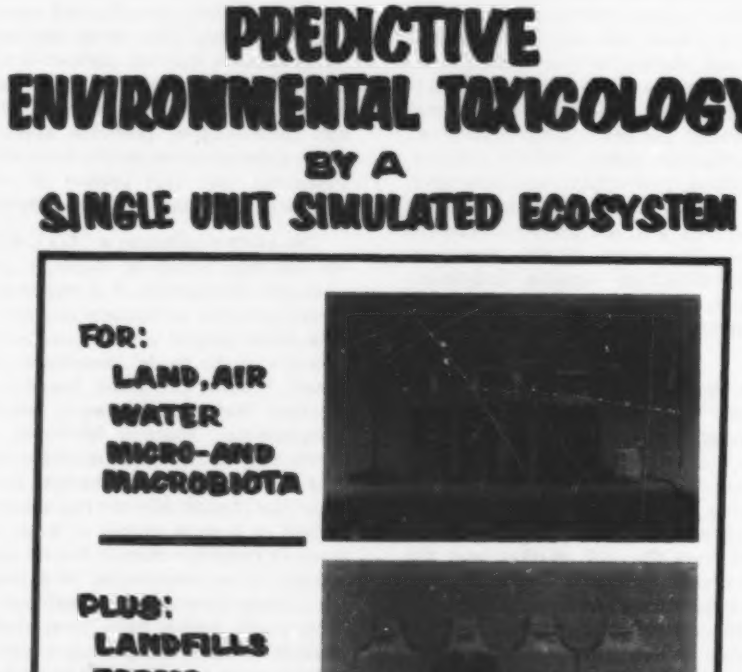


Figure 41. Physical model ecosystem; (top) arrangement for periodically monitoring volatile components and for evaluating leaching and run-off; (bottom) terrestrial and aquatic sequential phases.

air. Each carboy contained either 400 g of vermiculite (Terra-Lite, W. R. Grace and Co., Cambridge, Mass.) or 3000 g of Drummer silty clay loam soil (particle diameter 2.4 - 4.8 mm). The general soil characteristics were: 9.5% sand, 60.2% silt, 30.3% clay, and 7.1% organic matter. Fifty corn seeds (*Zea mays*, Pioneer Hi-Bred 3334A, St. Joseph, Ill.) were uniformly planted in each carboy at a depth of one cm. For preemergent application of a pesticide (Figure 42), a total of 5 mg of radiolabeled compound in one ml of acetone was injected (20 μl per injection) at a depth of one cm beside each of the seeds. For postemergent application, the same amount of compound in one ml of acetone was applied to each plant (20 μl per plant) ten days after planting the system. This rate simulated a one pound active ingredient per acre application, which is the commonly used treatment rate in the field. Vermiculite ecosystems were sprinkled with two liters, and soil ecosystems with one liter, of Freeman's standard reference water (Freeman, 1953). Each system was weighed after planting and watering, and again 7 and 14 days later at which times enough distilled water was added with a sprinkler to restore the initial wet weight, thus simulating two rains and compensating for the evaporation of water.

The following invertebrate animals were added to the system on Day 10: *Estigmene acrea* (Drury), the saltmarsh caterpillar; *Limax maximus* L., a garden slug; *Lumbricus terrestris* L., an earthworm; and *Armadillidium vulgare* (Latreille), a pillbug. Samples of each invertebrate species were analyzed for pesticide residue on Day 15, at which time the prairie vole, *Microtus ochrogaster* (Wagner), was added to the system. It was supplied with a cup of fresh water daily, and removed for analysis on Day 20. Any readily visible plant debris and invertebrate animals remaining in the terrestrial phase were removed on Day 20 and discarded; the substrate was then mixed well and sampled for analysis. After substrate sampling on Day 20, the ecosystem was flooded with 7 liters of standard reference water, and snails (*Physa* sp.), mosquito larvae (*Culex pipiens quiquefasciatus*) and water fleas (*Daphnia magna*) were added. Three fish (Gambusia affinis) were added on Day 24, and all aquatic organisms were removed for analysis on Day 27. The water was decanted at this time. The wet sediment was left in the container for 1 or 5 days and them mixed well and sampled for analysis. The ecosystem units were housed in a 244 x 132 x 198-cm walk-in environmental plant growth chamber (PGW 36, Controlled Environments, Pembina, N. Dak.) which was operated at the following parameters: light/dark phase, 12:12 hours; continuous air circulation; day air temperature, 26°C; night air temperature, 19°C; day substrate temperature, 24-26°C; night substrate temperature, 20-21°C; relative humidity, 50%; light intensity at substrate surface, 1000 foot-candles.

Air was routinely monitored for a three-hour daylight period at a flow rate of 10 ml/sec on the days designated in Figure 42. The inlet tube of the ecosystem carboy was connected by means of silicone rubber tubing, fitted with a needle valve, to a Silent Giant® aquarium pump (Aquarium Pump Supply, Inc., Prescott, Ariz.) providing a slight positive pressure within the carboy, which "pushed" the air from the carboy to the gas traps which were connected in series and terminated with a flow meter. The gas traps consisted of two 125-ml gas washing bottles containing glass tubing which terminated in fritted glass cylinders of extra course porosity. The two bottles were mounted in Dewar flasks packed with crushed ice. (The vapor trap preceding the two gas traps, shown in Figure 41, was not used in these experiments; it was used only in concomitant studies involving long-term gas trapping.) The first trap (closest to the ecosystem carboy) contained 75 ml of acetonitrile, and the second trap contained 75 ml of a solution of ethanolamine and 2-methoxyethanol (1:2 by volume). The volatile radioactive products trapped in these solutions were quantified by direct scintillation counting using the scintillation fluid, Aquasol® (New England Nuclear), and a Packard Tri-Carb Model 3320 scintillation counter.

Descriptions of the analyses of the biological components and substrates have recently been presented (Cole et al., 1976a,b) and only a synopsis follows. The animal and plant samples were homogenized with ca. 20 volumes of cold acetone per volume of sample in a Sorvall Omni-Mixer or a glass tissue homogenizer, centrifuged, and the supernatant decanted. The pellet was again extracted with acetone using a vibrating-type mixer, and centrifuged. The second supernatant was combined with the first, forming the "extract" of the sample, an aliquot of which was analyzed for total radioactivity. The acetone-unextractable radioactivity remaining in the pellet described above was determined by dissolving a wet pellet in an equal volume of Protosol® (New England Nuclear) for 48 hrs in a closed vial at 50°C; an aliquot was subsequently counted in Aquasol®. The counts from the pellet were added to those from the extract to

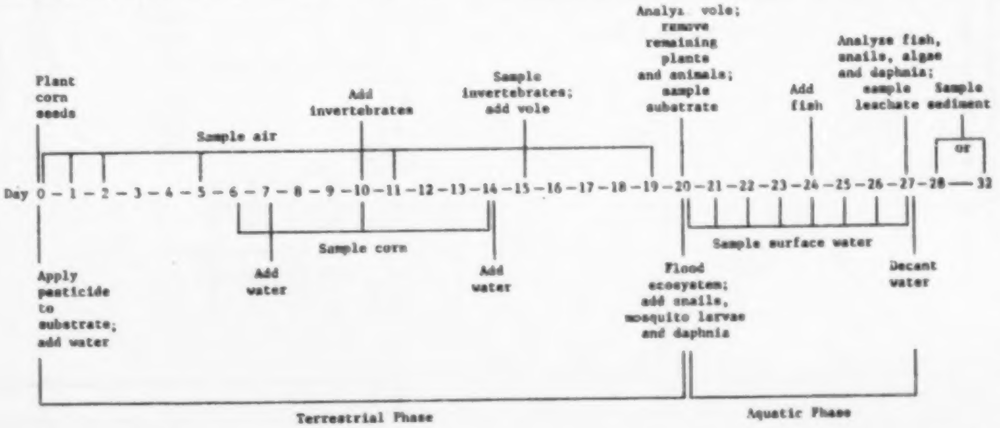

Plant corn seeds
Sample air
Add invertebrates
Sample invertebrates; add vole
Analyz vole; remove remaining plants and animals; sample substrate
Add fish
Analyze fish, snails, algae and daphnia; sample leachate
Sample sediment
or
Day 0 – 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 – 11 – 12 – 13 – 14 – 15 – 16 – 17 – 18 – 19 – 20 – 21 – 22 – 23 – 24 – 25 – 26 – 27 – 28 — 32
Sample corn
Sample surface water
Apply pesticide to substrate; add water
Add water
Add water
Flood ecosystem; add snails, mosquito larvae and daphnia
Decant water
Terrestrial Phase
Aquatic Phase

Figure 42. Time sequence for the model ecosystem when using a preemergent substrate pesticide application: postemergent foliar applications are made at day 10, the air-monitoring is shifted to days 10, 11, 12, 15 and 19, and the corn is sampled on days 12 and 14.

determine the total radioactivity of the sample. Soil (or vermiculite) was homogenized as above in acetone, and subsequently vacuum-filtered. The filter cake was then homogenized in methanol and vacuum-filtered. The acetone and methanol filtrates were counted individually, and then summed to obtain the extractable radioactivity. The unextractable radioactivity (for 5 of the soil ecosystems) was determined by combustion of filter cake aliquots. Samples of water were counted directly in Aquasol®. The separation, identification, and quantification of metabolites were carried out by means of thin-layer chromatography on silica gel, followed by autoradiography and subsequent scraping of radioactive TLC spots into scintillation vials for counting.

The following ^{14}C-labeled pesticides were ring-labeled, except for captan which was labeled at the trichloromethyl carbon and fonofos which was labeled at the O-ethyl carbon. The final adjusted specific activity (mCi/mmol) is shown in parentheses following the common name, which precedes the chemical name. PCNB, PCP, and captan are fungicides; the others are insecticides. Fifty μCi were added to an individual ecosystem.

Methoxychlor (1.61): 1,1,1-trichloro-2,2-*bis*(p-methoxyphenyl)ethane

DDT (3.54): 1,1,1-trichloro-2,2-*bis*(p-chlorophenyl)ethane

Fonofos (2.70): *O*-ethyl *S*-phenyl ethylphosphonodithioate

Aldrin (3.10): 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-*endo*-1,4-*exo*-5,8-dimethanonaphthalene

Dieldrin (3.68): 1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene

PCNB or quintozene (4.32): pentachloronitrobenzene

PCP (2.55): pentachlorophenol

Parathion (4.16): *O,O*-diethyl *O*-p-nitrophenyl phosphorothioate

Methyl parathion (5.60): *O,O*-dimethyl *O*-p-nitrophenyl phosphorothioate

Captan (2.99): *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide

RESULTS AND DISCUSSION

The coherent environmental behavior of a given pesticide and its derivatives throughout an individual model ecosystem is shown in Figures 43-47 for each of the ten pesticides investigated. In examining a particular system, it is important to note whether the pesticide was applied as a preemergent application to soil at the time of planting (Day 0), or as a postemergent application to corn foliage ten days after planting. It is also important to note the type of substrate in each system. For each pesticide, it was obvious that its accumulation and/or degradation was not equal in various species. Samples of all terrestrial animals were examined after a five-day exposure. An aliquot of corn was always analyzed (shoots and roots separately) 14 days after planting, and at additional times in certain systems. Fish and snails were analyzed after a three-day and seven-day exposure, respectively. A pesticide characteristic in a vole refers to the entire vole (not just some specific tissue) unless otherwise specified; the same is true for all other animals and plants.

DDT, aldrin and dieldrin (Figures 43, 44 and 45) are considered as unduly hazardous pesticides and have been removed from the U.S. market by the U.S. Environmental Protection Agency. Thus, the reader may especially wish to compare the behaviors of the other compounds to these. At the other extreme, our studies indicated that fonofos, captan, and methyl parathion (Figures 43, 45 and 47) were the most environmentally labile of the various compounds investigated. But it should be borne in mind that our current sample size of ten compounds is quite small compared to the approximately 1,500 pesticide active ingredients (Chem. and Engineering News, 1976) now in existence.

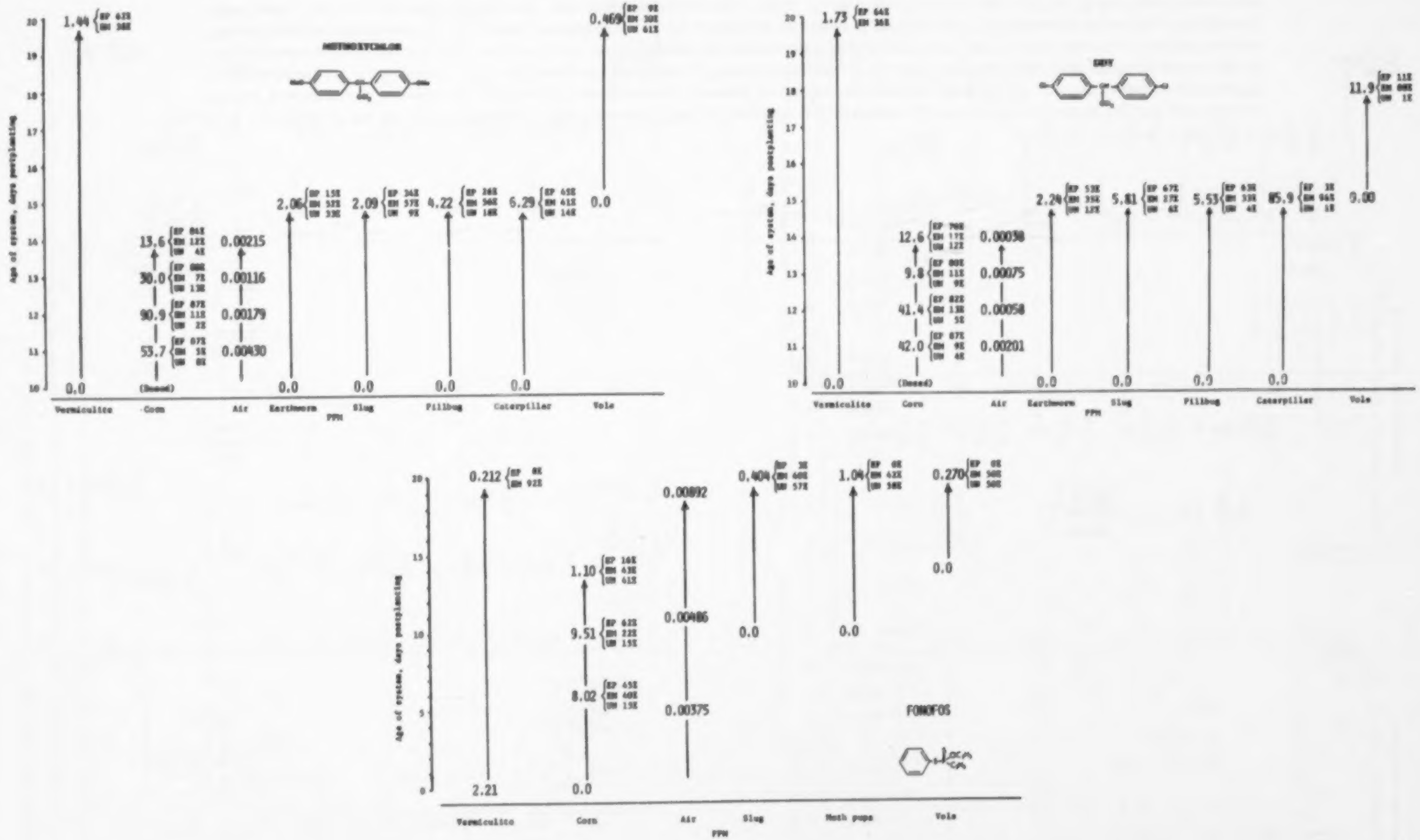


Figure 43. Summary of the fates of [^{14}C]methoxychlor, [^{14}C]DDT and [^{14}C]fonofos in vermiculite-terrestrial model ecosystems. The total ^{14}C-residues are expressed as parent compound equivalents, ppm (w/w), and their subdivisions are EP = extractable parent compound, EM = extractable metabolites of the parent compound, and UN = unextractable products remaining in the processed sample. On day zero, the corn seeds were planted, and the vermiculite of the fonofos system was treated with the pesticide at one lb AI/acre. On day 10, the corn foliage of the methoxychlor and DDT systems was treated randomly with the appropriate pesticide at one lb AI/acre, and the invertebrates were added to each system. On day 14, the vole was added to the fonofos system; the voles were added to the methoxychlor and DDT systems on day 15.

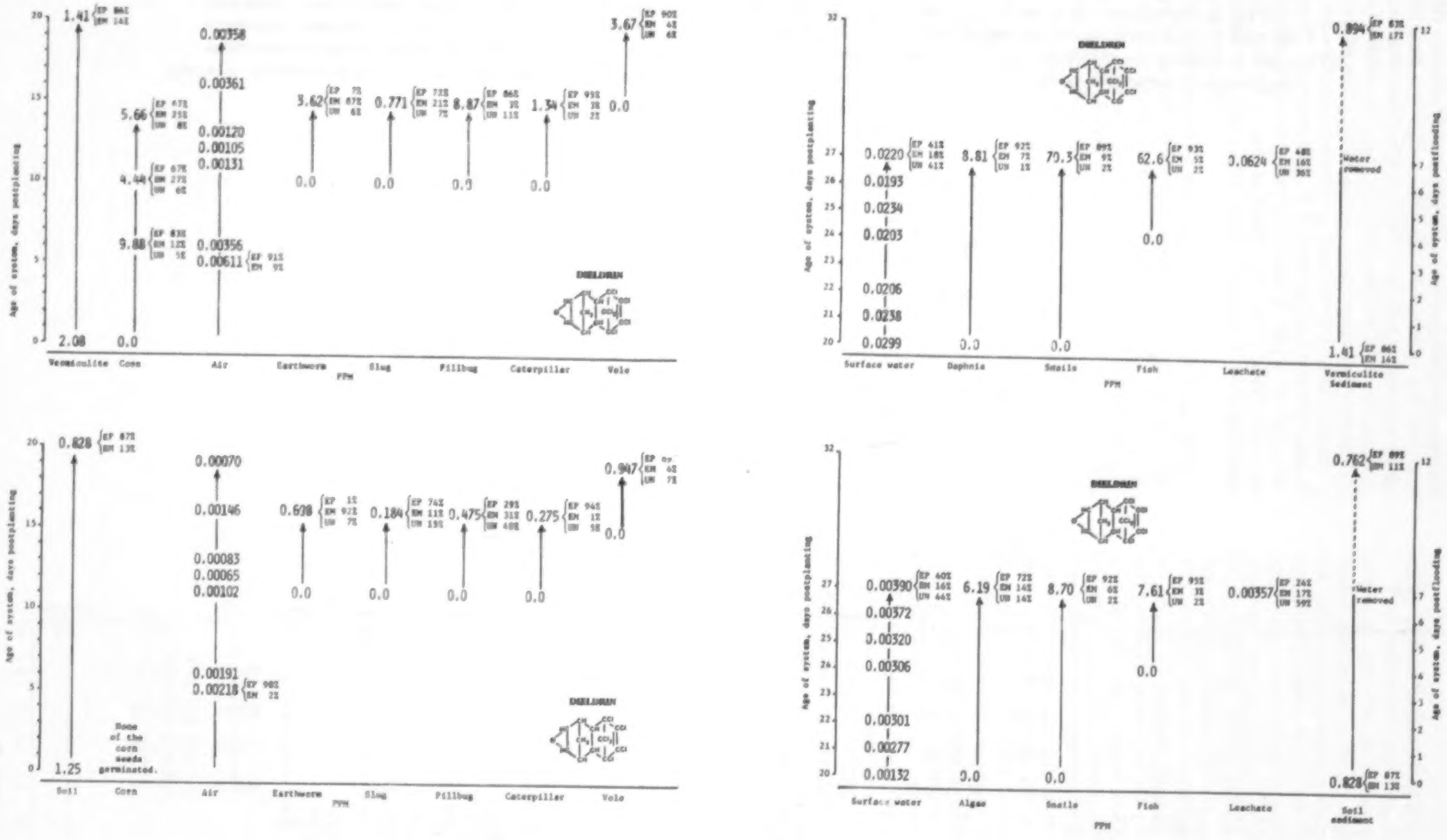


Figure 44. Summary of the fate of [^{14}C]dieldrin in the terrestrial (left) and aquatic (right) phases of vermiculite-terrestrial (upper) and soil-terrestrial (lower) model ecosystems. The total ^{14}C-residues are expressed as dieldrin equivalents, ppm (w/w), and their subdivisions are EP = extractable parent compound, EM = extractable metabolities of the parent compound, and UN = unextractable products remaining in the processed sample. On day zero, the corn seeds were planted, and the vermiculite and soil were treated with dieldrin at one lb AI/acre. The soil system was drenched with water, instead of the usual sprinkling, and none of the corn seeds germinated; extraneous foliage was added later. The invertebrates and the vole were added to each system 10 and 15 days later, respectively. On day 20, the barren terrestrial phase of each system was flooded, and snails, Daphnia and mosquito larvae were added; the fish were added 4 days later; the algae originated in the soil.

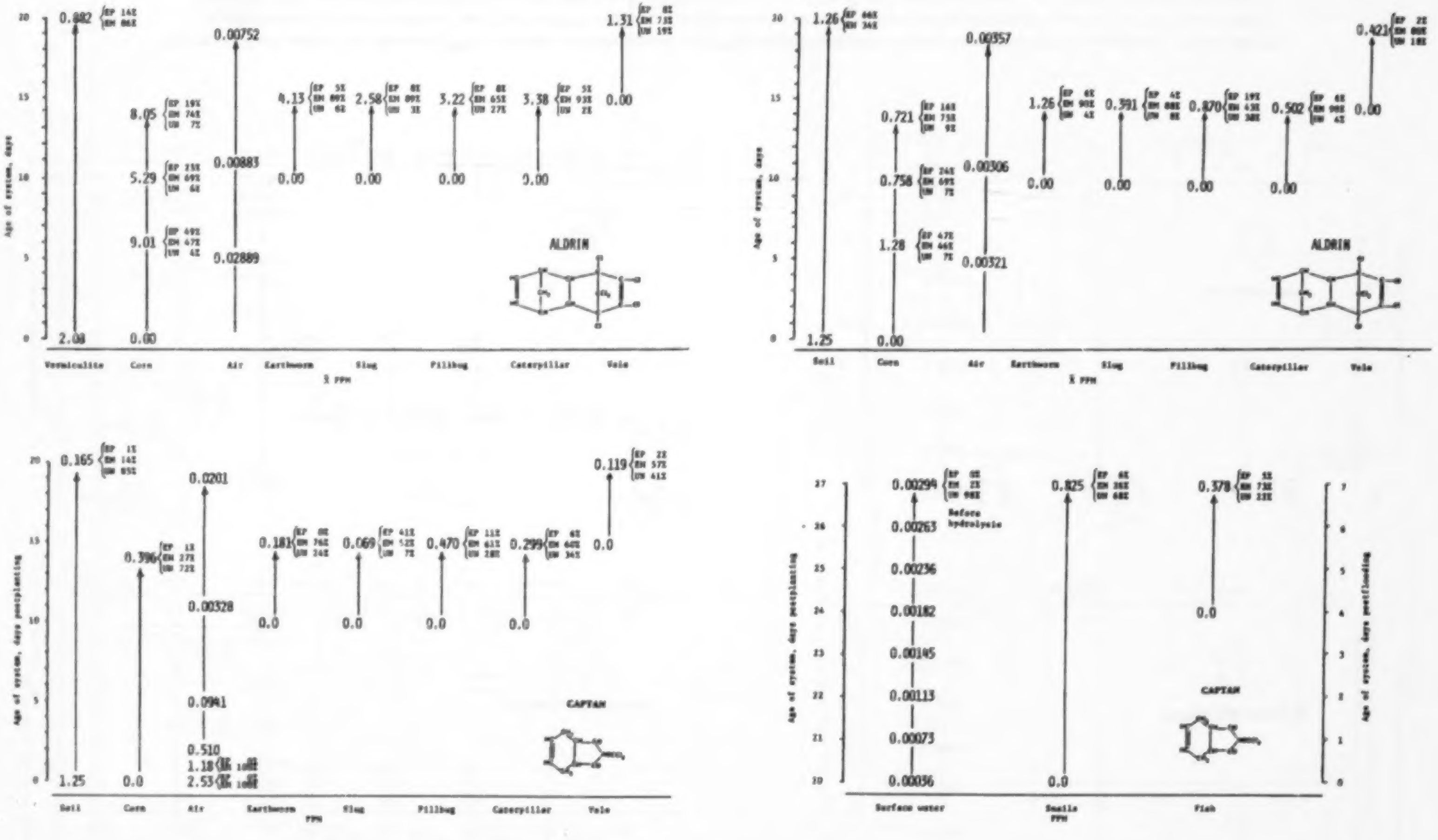


Figure 45. Summary of the fates of [14C]aldrin in vermiculite-terrestrial (upper left) and soil-terrestrial (upper right) model ecosystems and [14C]captan in a soil-terrestrial model ecosystem. The total 14C-residues are expressed as parent compound equivalents, ppm (w/w), and their subdivisions are EP = extractable parent compound, EM = extractable metabolites of the parent compound, and UN = unextractable products remaining in the processed sample. On day zero, the corn seeds were planted, and the vermiculite and soil were treated with the appropriate pesticide at one lb AI/acre. The invertebrates and the vole were added to each system on days 10 and 15, respectively. On day 20, the barren terrestrial phase of the captan system was flooded, and snails, Daphnia and mosquito larvae were added; the fish were added 4 days later.

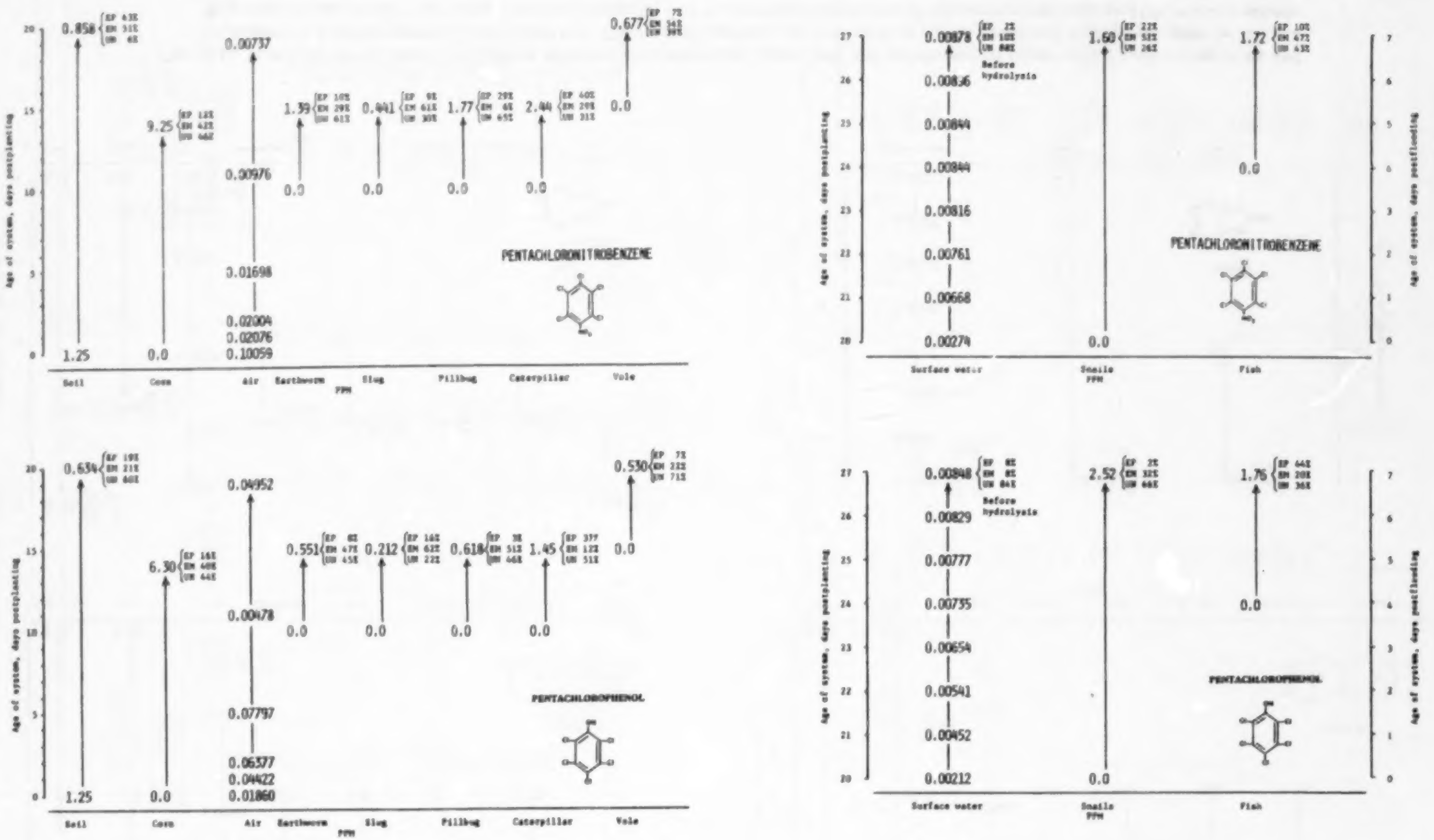


Figure 46. Summary of the fates of [^{14}C]pentachloronitrobenzene and [^{14}C]pentachlorophenol in the terrestrial (left) and aquatic (right) phases of soil-terrestrial model ecosystems. The total ^{14}C-residues are expressed as parent compound equivalents, ppm (w/w), and their subdivisions are EP = extractable parent compound, EM = extractable metabolites of the parent compound, and UN = unextractable products remaining in the processed sample. On day zero, the corn seeds were planted, and the soil in each system was treated with the appropriate pesticide at one lb AI/acre. The invertebrates and the vole were added to each system on days 10 and 15, respectively. On day 20, the barren terrestrial phase of each system was flooded, and snails, Daphnia and mosquito larvae were added; the fish were added 4 days later.

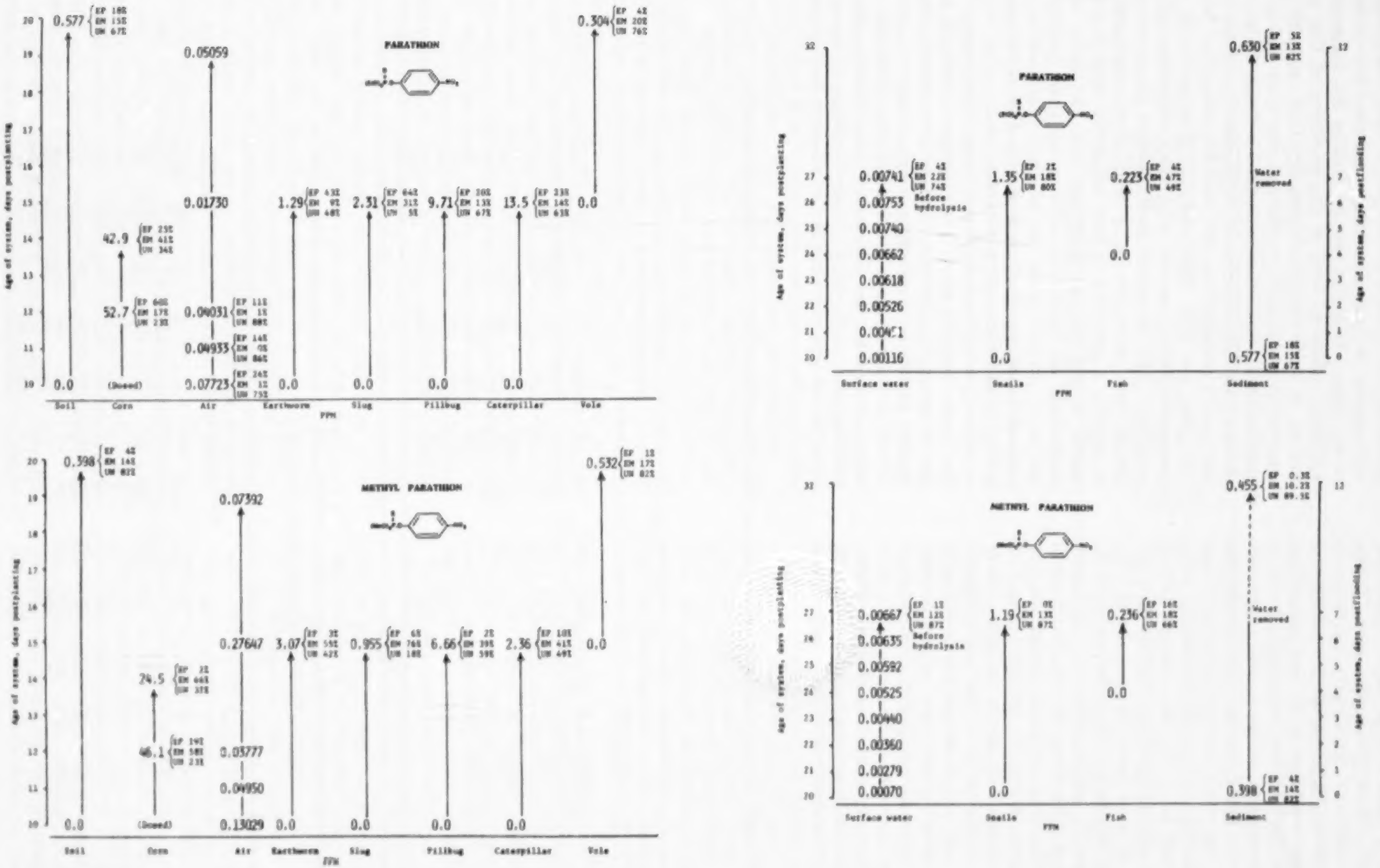


Figure 47. Summary of the fates of [^{14}C]parathion and [^{14}C]methyl parathion in the terrestrial (left) and aquatic (right) phases of soil-terrestrial model ecosystems. The total ^{14}C-residues are expressed as parent compound equivalents, ppm (w/w), and their subdivisions are EP = extractable parent compound, EM = extractable metabolites of the parent compound, and UN =unextractable products remaining in the processed sample. On day zero, the corn seeds were planted. On day 10, the corn foliage was treated with the appropriate pesticide at one lb AI/acre, and the invertebrates were added to each system; the vole was added on day 15. On day 20, the barren terrestrial phase of each system was flooded, and snails, Daphnia and mosquito larvae were added; the fish were added 4 days later.

In order to facilitate the comparisons of a great deal of values, we have made a number of pertinent summaries. Three of these are referred to as "grand summaries," Tables 13, 14 and 15. Many additional calculations can be easily made from these tables—but with caution. For example, in Table 15, if you average the values in the row across from the heading "vole," you find that the average percent of extractable parent compound detected in the total ^{14}C-pesticidal residues examined in voles was 19%. But for dieldrin, 89% of the residue in voles was parent compound, and there was no parent compound in the total fonofos residue in the vole. The above average value may be of some help in simplifying the data; it certainly indicates that less than 20% of the pesticide residues were parent compounds in voles. But it is also a good idea to glance at the entire row of values to note the extremes and the trends (medians), if any exist. In calculating an average value across the row, the mode of pesticide application, time of pesticide application, and type of terrestrial substrate are in essence ignored, but each of these characteristics substantially affects the behaviors of pesticides, as will be discussed later. The major subdivisions (extractable parent compound, extractable metabolites, and unextractable products) of each of the total ^{14}C-pesticide residue values summarized in Table 1 can be located by referring to Figures 43-47.

In Table 15, it is apparent that aldrin was readily metabolized in the vole, but it is not apparent from this table that the resulting major metabolite of aldrin was dieldrin in the vole. We have compiled more than 100 additional tables describing the detailed metabolism of each compound by each species, but those data are too numerous to be included in this report. However, we are making this information available in booklet form at the workshop of this symposium. In Table 15, it is obvious that DDT (as such) was also readily metabolized by the vole. But if we look at the entire column of values beneath DDT in Table 15, we note that the only other component of the model ecosystem that also readily metabolized DDT was the caterpillar. In the caterpillar and vole, DDT was readily converted to predominately DDE; however, in all other components of the ecosystem, DDT remained predominately as parent compound. Thus, when examining these "grand summaries" it is not only a good idea to look across the rows, it is also useful to look up and down the columns. Even when ecosystems are examined under homologous conditions, and at a reduced scale, all of their complexities and differences are not eliminated. A vole does not become a pillbug, does not become a corn plant, etc.

Before leaving Table 15, if one averages the values in the row across from the heading "earthworm," one finds that, on the average, earthworms appear to metabolize these specific pesticides more efficiently than any of the other terrestrial animals examined. But did the metabolism actually occur within the earthworms, or in the substrate surrounding the earthworms? It very probably occurred in both, but if you compare the earthworm and substrate

Table 13—Grand summary no. 1: total ^{14}C-pesticidal residues, ppm, in terrestrial-modular model ecosystems.

Ecosystem component and postplanting age at analysis (days)	Vermiculite substrate				Soil (silty clay loam) substrate							
	Postemergent application			Preemergent application							Postemergent application	
	Methoxychlor	DDT	Fonofos	Dieldrin	Aldrin	Aldrin	Dieldrin[a]	PCNB	PCP	Captan	Parathion	Me parathion
Substrate (20)[b]	1.44	1.73	0.212	1.41	0.882	1.26	0.828	0.858	0.634	0.165	0.577	0.398
Air (5 or 15)	0.002	0.0004	0.004	0.006	0.029	0.003	0.002	0.017	0.078	0.094	0.017	0.276
Corn (14)	13.6	12.8	1.10	5.66	8.05	0.721	...	9.25	6.30	0.396	42.9	24.5
Earthworm (15)	2.06	2.24	...	3.62	4.13	1.28	0.686	1.39	0.551	0.181	1.29	3.07
Slug (15)	2.08	5.01	0.404	0.771	2.58	0.391	0.184	0.441	0.212	0.069	2.31	0.955
Pillbug (15)	4.22	5.53	. . .	8.87	3.22	0.870	0.475	1.77	0.018	0.470	9.71	6.66
Caterpillar (15)	6.29	85.9	1.04	1.34	3.38	0.502	0.275	2.44	1.45	0.299	13.5	2.36
Vole (20)	0.469	11.9	0.270	3.67	1.31	0.421	0.947	0.677	0.530	0.119	0.304	0.532
Water (27)				0.022			0.004	0.009	0.008	0.003	0.007	0.007
Sediment (32)[b]				0.894			0.762	...	...	...	0.630	0.455
Snail (27)				70.3			8.70	1.80	2.52	0.825	1.35	1.19
Fish (27)				62.6			7.01	1.72	1.78	0.378	0.223	0.236

[a] No corn plants were present in this model ecosystem.

[b] For Methoxychlor, DDT, Fonofos, Dieldrin and Aldrin, value represents extractable ^{14}C only; for Aldrin, 100% of the ^{14}C-pesticide was extractable.

Table 14—Grand summary no 2: extractable parent ^{14}C-pesticidal compounds, ppm, in terrestrial-modular model ecosystems.

Ecosystem component and postplanting age at analysis (days)	Vermiculite substrate				Soil (silty clay loam) substrate							
	Postemergent application			Preemergent application							Post emergent application	
	Methoxychlor	DDT	Fonofos	Dieldrin	Aldrin	Aldrin	Dieldrin[a]	PCNB	PCP	Captan	Parathion	Me parathion
Substrate (20)	0.886	1.12	0.016	1.21	0.125	0.840	0.722	0.369	0.119	0.002	0.104	0.015
Air (5 or 15)[b]	0.002	0.0004	0.004	0.006	0.020	0.003	0.002	0.017	0.078	0.094	0.017	0.276
Corn (14)	11.4	9.57	0.175	3.80	1.55	0.115	...	1.88	1.01	0.004	10.9	0.586
Earthworm (15)	0.316	1.18	...	0.225	0.223	0.070	0.011	0.146	0.046	0.0	0.556	0.092
Slug (15)	0.700	3.87	0.012	0.554	0.182	0.012	0.137	0.042	0.035	0.028	1.47	0.053
Pillbug (15)	1.10	3.48	...	7.50	0.247	0.086	0.138	0.511	0.022	0.053	1.97	0.150
Caterpillar (15)	2.83	2.86	0.0	1.26	0.180	0.032	0.257	0.973	0.541	0.019	3.19	0.224
Vole (20)	0.041	1.37	0.0	3.29	0.150	0.008	0.843	0.051	0.037	0.002	0.013	0.005
Water (27)				0.009			0.002	0.0002	0.001	<0.0001	0.0003	0.0001
Sediment (32)				0.745			0.079	...	...	...	0.029	0.0013
Snail (27)				62.9			8.02	0.390	0.048	0.048	0.030	0.0
Fish (27)				58.1			7.25	0.171	0.779	0.021	0.008	0.038

[a] No corn plants were present in this model ecosystem.

[b] Total ^{14}C-pesticidal residue.

Table 15—Grand summary no. 3: resistance to degradation, expressed as the percent of extractable parent compound present in the total ^{14}C-pesticidal residue

Ecosystem component and postplanting age at analysis (days)	Vermiculite substrate					Soil (silty clay loam) substrate						
	Postemergent application					Preemergent application					Postemergent application	
	Methoxy-chlor	DDT	Fonofos	Diel-drin	Aldrin	Aldrin	Diel-drin[a]	PCNB	PCP	Captan	Para-thion	Me para thion
Substrate (20)	61.53	64.74	7.55	85.82	14.17	66.67	87.20	43.01	18.77	1.09	18.02	3.77
Air (5 or 15)												
Corn (14)	83.82	75.95	15.91	67.14	19.25	15.95	...	11.68	16.03	1.01	25.41	2.43
Earthworm (15)	15.34	52.68	...	7.04	5.40	5.56	1.58	10.50	8.35	0.0	43.10	3.00
Slug (15)	33.49	66.61	2.97	71.85	7.05	3.07	74.46	9.52	16.51	40.58	63.64	5.55
Pillbug (15)	26.07	62.93	...	85.57	7.67	10.92	29.05	28.87	3.56	11.28	20.29	2.25
Caterpillar (15)	44.98	3.47	0.0	94.03	5.33	6.37	93.45	39.88	37.31	6.35	23.63	9.49
Vole (20)	8.74	11.51	0.0	89.65	11.45	1.90	89.02	7.53	6.98	1.68	4.28	0.94
Water (27)				40.85			40.26	1.71	8.14	0.10	3.91	1.35
Sediment (32)				83.33			88.98	...	...	...	4.67	0.29
Snail (27)				89.47			92.18	21.88	1.83	5.82	2.22	0.0
Fish (27)				92.81			95.27	9.94	44.26	5.56	3.59	16.10

[a]No corn plants were present in this model ecosystem

values for each chemical listed in Table 15, it will be noted for all chemicals except one (parathion) that the proportion of parent compound in earthworms was always less than that in the surrounding substrate, implying that more catabolism occurred in earthworms than in the surrounding substrate; this was apparent whether the substrate was vermiculite or Drummer silty clay loam soil.

The more stable compounds were no more readily degraded in soil than in animals. Aldrin and dieldrin were tested in both vermiculite and soil; there was no indication that either of these compounds was more readily degraded in soil containing a rich microflora than in vermiculite. If anything, it appeared that aldrin was more readily degraded in vermiculite than in soil; the ratio of parent compound to total residue was certainly less in vermiculite than in soil. But we really do not know whether these final ratios in substrate were due primarily to degradation that occurred within the substrate itself, or to degradation that occurred principally elsewhere within the ecosystem. Since the substrate is the most massive component of the model ecosystem, it probably is a reflection of the degradation that occurred in the substrate. But we also have to keep in mind that the final ratio of parent compound to total residue in substrate was not solely the result of degradation. Differences in the evaporation rates of parent compound and metabolites could also have easily altered the ratio of parent compound to total residue in the substrate. This was quite possible in the case of aldrin applied to vermiculite, because it was estimated that 53% of the total residue was lost to the atmosphere after 20 days. But in the vermiculite-containing system treated with dieldrin, only 27% of the total residue was lost to the air after the same period of time. Also, it is known that the vapor pressure of aldrin *in vitro* is greater than that of dieldrin. Thus, in the system containing vermiculite and treated with aldrin, the small ratio of parent compound to total residue may have resulted because aldrin more readily evaporated from the substrate than did its major metabolite, dieldrin. This is an excellent example of the interdependence of the parameters, degradation and environmental distribution; these measurements are the results of a combination of processes working together simultaneously. But the meaningful point is that the final outcome (parent compound/total residue in substrate) of interacting processes was readily detected and quantified using a model ecosystem.

In another model ecosystem treated with aldrin—the system containing soil—it is very improbable that transportation to the atmosphere was the cause of the change in the ratio of parent compound to total residue in the substrate, because no significant quantity of the residue evaporated. In the aldrin-soil system, the change was truly caused principally by degradation; in the aldrin-vermiculite system, it is very probable that the major portion of the change was caused by evaporation. We are going to great lengths to try and stress that although a given table, such as Table 15, may have a single parameter as its heading—such as "degradation"—that does not mean that multiple processes did not affect that measurement or parameter. It is very important to note that the relative degrees of degradation (transformation) and distribution (transportation) are not always easy to measure independently in open systems. Toxicologists who have tried to separate degradation from penetration (transport across membranes) have recognized this for a long time. Fundamental phenomena are often the results of very interdependent processes in any living organism or complex system. Degradation, accumulation, and distribution are fundamentals in the attempts to decipher the environmental fates of toxicants.

Environmental Distribution

To ascertain if a toxic substance is likely to be harmful, you have to know where it is likely to be, and to what extent. The distinctly different distributions of the various pesticides and their derivatives among the major environmental compartments of the model ecosystems at the end of the 20-day terrestrial phase are summarized in Figure 48. The detailed explanation as to how a given pesticide's distribution was calculated is exemplified in Figure 49.

The major relocation site of all pesticides was either the land or the atmosphere. For those compounds which were applied directly to Drummer silty clay loam soil, the maximum amount of any pesticide plus its derivatives that was detected in the total complement of terrestrial animals was 2% of the applied dose (aldrin and PCNB); the minimum amount was 0.2% (captan). Parathion and methyl parathion were carefully and directly applied to corn grown in soil ten days after planting; the respective amounts of their total residues in the total terrestrial animal masses were 4% and 3% of their applied doses. Thus it appeared that the compounds which were carefully applied (not sprayed) to corn foliage could produce twice as much contamination of the total animal complement after a short period of time than those compounds which were applied to soil. But at this time, we do not know whether the apparent differences in results were primarily due to differences in application techniques, or to differences in pesticides. In comparing parathion with aldrin, and taking into consideration that they are normally applied as simulated, we would conclude

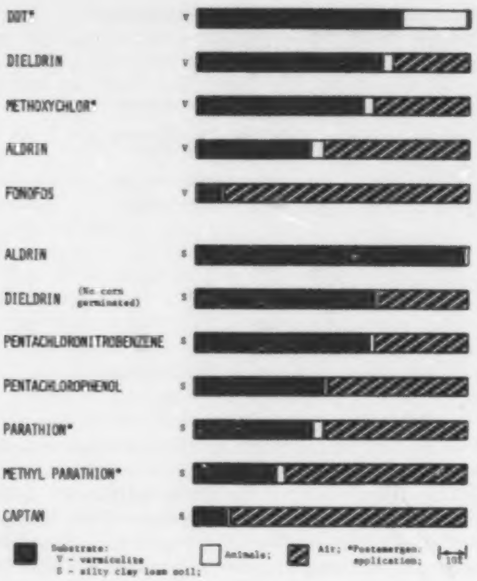
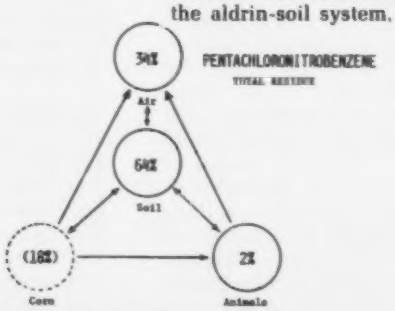


Figure 48. Environmental distributions of the total ^{14}C-pesticidal residues (% of the applied dose) at the termination of the terrestrial phase of various model ecosystems dosed with a given pesticide. The plants, having been consumed and/or demolished by the animals, are not present as an entity at the termination of the system. (Calculations and more detailed distributions are presented in the test.)

that the long-term, or chronic, potential hazard of parathion to the terrestrial animals in the agricultural environment would be much less than that of aldrin, because 52% of the parathion application was lost to the atmosphere after 10 days, whereas virtually none of the aldrin was lost to the atmosphere after 20 days. As indicated in Figure 48, methyl parathion would be potentially less hazardous than parathion since less of it was transferred to the animals and less remained in the soil. These compounds are presently being compared on the basis of their distributions; their relative degradations and accumulations will be presented later. Their known acute toxicities to animals will not be discussed, but of course these would be taken into consideration in the overall evaluation of their chronic potential hazards.

Figure 48 clearly indicates that the chronic potential hazard of the three fungicides would be in the order PCNB > PCP > captan. Their respective transfers to the total terrestrial animal complements were 2%, 1%, and 0.2% of their applied doses. Their respective persistences in soil after 20 days were 64%, 48% and 13% of their applied doses.

A change in the type of terrestrial substrate drastically altered the environmental distribution of a given pesticide as is evident by comparing the distributions of aldrin applied to vermiculite with that applied to Drummer silty clay loam soil (organic matter content 7%), as shown in Figure 48. The values shown are the averages of duplicate systems containing vermiculite and duplicate systems containing soil. When applied to vermiculite, the resulting major site of pesticide contamination was the atmosphere; when applied to soil, the vast majority of the total aldrin residue remained within the soil. Evidently, aldrin was less tightly bound to vermiculite than to soil. Each species of terrestrial animal in the aldrin-vermiculite system accumulated a greater concentration of total aldrin residue than did its counterpart in the aldrin-soil system, and the same relative relationship



Calculation of the above estimates:

Ecosystem component	Total maximum mass, g:			Mean concentration, µg/g	Total residue, µg:	Total residue as a % of applied dose (5000 µg)
	(No.)	$\overline{X}$ mass	= total		(mass)(conc.)	
Earthworms	(8)	3.36	= 26.9	1.39	37.4	0.7
Slugs	(4)	9.09	= 36.4	0.441	16.0	0.3
Pillbugs	(13)	0.0333	= 0.433	1.77	0.766	<0.1
Caterpillars	(8)	0.890	= 7.12	2.44	17.4	0.3
Vole	(1)	25.5	= 25.5	0.677	17.3	0.3
Animal total (I)			96.4		88.9	1.6
Corn[a/]	(28)	3.39	= 94.9	9.25	878	17.6
Soil (II)	(1)	3718	= 3718	0.858	3190	63.8
Air				[5000 µg-(I+II)]	= 1721	34.4

[a/] Proportion at 14 days postplanting; the corn, having been consumed and/or demolished by the animals, is not present as an entity at the termination of the system.

Figure 49. Terminal environmental distribution of [^{14}C]pentachloronitrobenzene plus metabolites in a soil-terrestrial model ecosystem.

was even more pronounced in the corn from the two systems (Table 13).

For the model ecosystems containing vermiculite, the aldrin-treated system is directly comparable to the dieldrin-treated system because they were homologous in all detail except for the type of pesticide (Figure 48). In comparing these two systems, it will be noted that aldrin was more volatile than dieldrin. However, in those systems containing soil, more dieldrin was lost to the atmosphere than aldrin; the probable cause of this was the absence of a growing crop in the dieldrin-soil system. This latter system was accidentally drenched with the proper amount of water (instead of it being slowly sprinkled) which compacted the soil and prevented the seeds from germinating. We do not believe that the complete lack of germination was due to the pesticide, because an aliquot of the same batch of seeds did germinate in the vermiculite-containing system treated with the same compound at the same rate. Soybean (*Glycine max*) and Swiss chard (*Beta vulgaris cicla*) leaves were added to the dieldrin-soil system to serve as food for the animals (corn leaves were not available). If a crop had grown in the dieldrin-soil system, we would have expected the retention of dieldrin in soil to be comparable to, or greater than, that of aldrin in soil. The root system of growing plants in essence contributes a tremendous quantity of organic matter to the soil environment; furthermore, it greatly aids in holding the soil together, thus preventing the formation of deep cracks or fissures which create abundant surface-to-air interfaces which enhance volatilization. It would seem strongly advantageous to have at least a growing crop present in those studies concerned with the fate of agricultural chemicals in agricultural soils. Although the volatilizations of dieldrin from vermiculite and from soil-without-a-crop were very similar, the accumulation of dieldrin residues by each animal species was much greater in the system with a growing crop. This implies that the volatilization, as such, was not nearly as significant a factor contributing to pesticide accumulation by animals as was the presence of a growing crop.

DDT and methoxychlor were applied directly to the corn growing in individual model ecosystems containing vermiculite (Figure 48). Six times more total DDT residue was transferred to the total animal complement than was total methoxychlor residue, and only 1% of the applied DDT dosage was transferred to the atmosphere ten days after application as compared to 35% for methoxychlor. This clearly indicated that the chronic potential hazard of DDT was much greater than that of methoxychlor.

Insecticidal Efficacies

The amount of total insecticide residue within the total complement of insects was always less than 1% of the applied dose for any given insecticide except DDT; 14.1% of the applied DDT dosage was detected as a residue in caterpillars, but 92% of this was DDE and the caterpillars had not been killed by the pesticide. Only 0.6% of the applied parathion was detected as a total residue (23% parent compound) in all caterpillars, but that was enough to have caused 88% of them to die. For methyl parathion, 0.35% of the applied dose was detected as a total residue (10% parent compound) in the total complement of caterpillars, but none had been killed. For methoxychlor, 0.99% of the applied dose was detected as a total residue (45% parent compound) in the total complement of caterpillars, but none had been killed. Clearly, the insecticidal efficacies of the various insecticides differed. The three compounds that were applied beneath the surface of the soil (aldrin, dieldrin, and fonofos) would not normally be used against this particular type of caterpillar, e.g., only 0.06% of the aldrin applied to soil was detected as a total residue in the total complement of caterpillars.

Pesticide Transport to Surface Water and Aquatic Organisms

At the termination of the terrestrial phase (Day 20), seven of the systems were each slowly flooded with seven liters of water, the mass of which was 1.75 times greater than that of the moist soil (Figure 50). This was certainly a harsh treatment of a land mass with water. Seven days after flooding the systems that contained a natural soil, the following amounts of total ^{14}C-pesticidal residues as a percent of the initial dose applied to the terrestrial phase, were detected in the waters: 1.2% PCNB, 1.2% PCP, 1% parathion, 0.9% methyl parathion, 0.5% dieldrin, and 0.4% captan. In the same respective order, the amounts detected in the total complement of aquatic organisms were: 0.04%, 0.05%, 0.02%, 0.02%, 0.30%, and 0.01%. For the system containing vermiculite and treated with dieldrin, 3.1% of the applied dose was transported to water, and 2.3% to aquatic organisms. Clearly, more total dieldrin residue was transported to the same volume of water from vermiculite than from soil. As noted earlier, the total mass of vermiculite differed from the total mass of soil; thus the concentrations of pesticides in the two substrates (Figure 50) are not directly comparable. The 1.41 ppm dieldrin residue in vermiculite represents an absolute amount of 3,384 μg; the comparable concentration and absolute amount in soil at the time of flooding were, respectively, 0.828 ppm and 3,312 μg. Thus, the absolute amounts of total dieldrin residues in vermiculite and

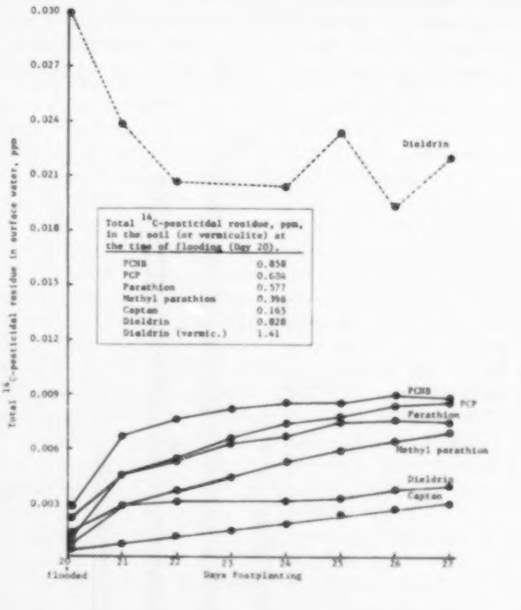


Figure 50. Translocations of total ^{14}C-pesticidal residues from soil (or vermiculite) to water. Solid lines: substrate was silty clay loam soil; dotted line: substrate was vermiculite. All systems were treated initially with the same absolute quantity of a given pesticide which in soil = 1.25 ppm, but in vermiculite = 2.08 ppm. The two parathions were applied initially to crop foliage, instead of substrate. No crop grew in the dieldrin-soil system.

soil were almost equal; yet approximately six times more pesticide residue was transported to the water in the vermiculite system than to the water in the soil system, indicating that the dieldrin residue was about six times more strongly bound to soil than to vermiculite under these conditions.

The amounts of pesticides transported from soil to surface water were quite small compared to the amounts transported from soil to air. PCNB and PCP were the two pesticides most abundantly transported to water from natural soil; 1.2% of the applied PCNB was transported to water over the seven-day period of flooding, whereas 34% was transported to the atmosphere during the preceding 20 days. Likewise, 1.2% of the applied PCP was transported to water, and 51% was transported to air. For captan, 0.04% of its applied dose reached water, whereas 87% was earlier lost to air. Thus, it was distinctly apparent that the amount of pesticide likely to reach water was very dependent upon the pesticide's past history on land, especially its volatilization from land. However, it was also noted that even though there were failrly large differences in the relative pesticide concentrations in soil at the time of flooding (Figure 50), the relative concentrations of different pesticides in water were clustered within a narrow range, ca. 3-9 ppb. This indicated that the eventual pesticide concentration in water was also a function of a given pesticide's relative mobility from soil to water (desorption and solubility).

As shown in Figure 51, an aquatic organism accumulated a tremendous quantity of pesticide from its surrounding aquatic environment, especially when compared to the general levels accumulated by terrestrial animals from their surrounding environment (Figure 52). For example, the concentration of total dieldrin residue in fish was 1,951 times greater than that found in the surrounding water, whereas the concentration in the vole was approximately the same as that detected in the surrounding soil (Table 13). For total PCP residues (Table 13), the following pesticide concentration ratios were noted: fish/water = 206, snail/water = 297, vole/soil = 0.836, and caterpillar/soil = 2.29. Recall the following exposure periods: 3 days for fish, 5 days for terrestrial animals, and 7 days for aquatic snails. Since the exposure period of the terrestrial animals was bracketed by those of the aquatic animals, there was no indication that the difference in exposure periods was responsible for the large difference in pesticide accumulations by aquatic and terrestrial animals. Clearly, the terrestrial environment is divided into many micro-compartments, it is not a fluid, and the accessibility of the total quantity of terrestrial pesticide to any given individual terrestrial organism is extremely limited. Metaphorically, we noted earlier that a vole was not a caterpillar; we would now like to note that it certainly is not a fish, and that a terrestrial environment is not an aquatic environment.

When attempting to assess the relative potential biological hazards of various pesticides in aquatic environments it would seem much more meaningful to compare their concentrations in some aquatic organism rather than their concentrations in water, because of the great differences among pesticides to be accumulated into the biotic component. For example, in Figure 51 note that the concentration of the total PCNB residue in water was much greater than that of the total dieldrin residue, but nevertheless a substantial inverse relationship was true for fish. And more importantly, unless a given pesticide is normally applied directly to water, the experimental assessments of the relative aquatic hazards of pesticides should begin with the applications of the compounds to the terrestrial environment, for all of the reasons discussed above. In addition, if the primary potential defilement of concern is some form of terrestrial life, it would certainly be more meaningful to study the terrestrial environment.

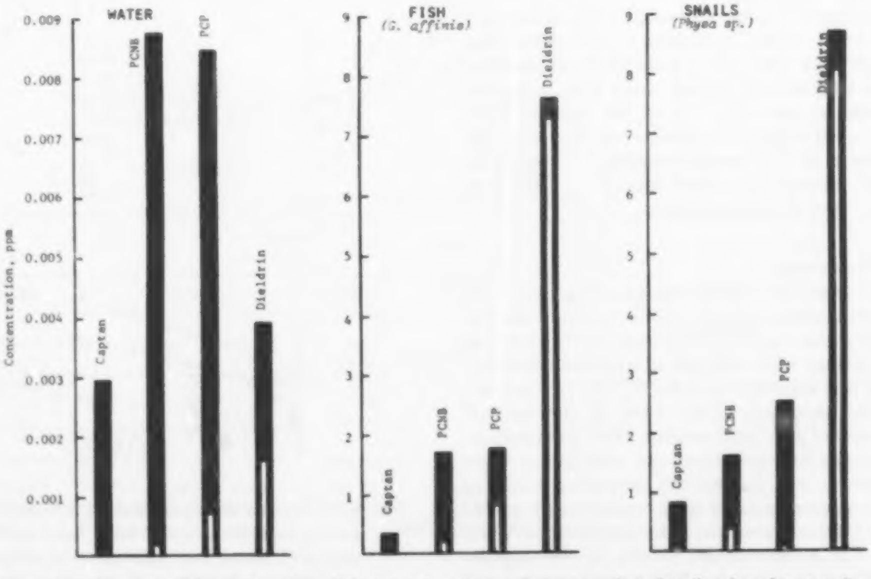


Figure 51. Total ^{14}C-pesticidal residues (black bars) and parent compounds (white bars) in the aquatic phase of a soil-terrestrial model ecosystem treated with [^{14}C]dieldrin compared with those treated with fungicides. The barren terrestrial phases were flooded 20 days after dosing the soils. The snails were added at the time of flooding, and the fish were added 4 days later; water, fish and snails were analyzed 7 days postflooding.

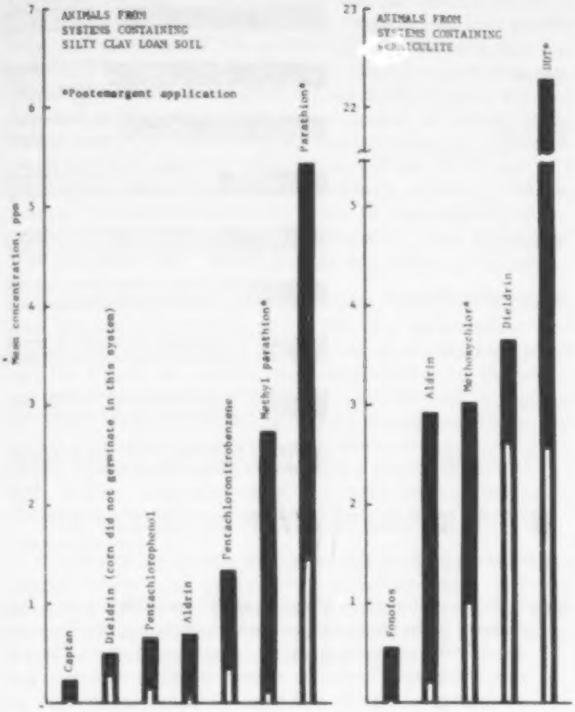


Figure 52. Accumulations of total ¹⁴C-pesticidal residues (black bars) and parent compounds (white bars) in terrestrial animals as averaged over 5 species (earthworms, slugs, pillbugs, caterpillars, and voles) from model ecosystems containing either a vermiculite substrate or a silty clay loam soil. The animals were analyzed after a 5-day exposure within a given model ecosystem. Each system was dosed with a single pesticide at a rate simulating one lb AI/acre; the mode of application was either preemergent to substrate or postemergent to foliage, as indicated.

Pesticide Accumulation and Degradation in Terrestrial Animals and Corn

Earlier we emphasized that fates of pesticides differed among various terrestrial animals. Species differences became more apparent when we compared results in terrestrial animals with those in aquatic animals. Aware of species differences, we averaged the pesticide accumulations (Figure 52) and degradation (Figure 53) that occurred in five species of animals, but note that they were the same five species for each pesticide. We have not yet tested different modes of pesticide application keeping the type of pesticide constant, but it currently appears that compounds that were applied directly to the crop were accumulated more extensively than those applied to the substrate. Substrate differences have been tested. Both aldrin and dieldrin were tested in two different substrates, and each was accumulated more by the animals within a vermiculite-containing system than by those in a system containing soil. Thus, in comparing the accumulations of various compounds in the present study, the most accurate comparisons are among those compounds which were applied in the same manner and to systems containing the same substrate.

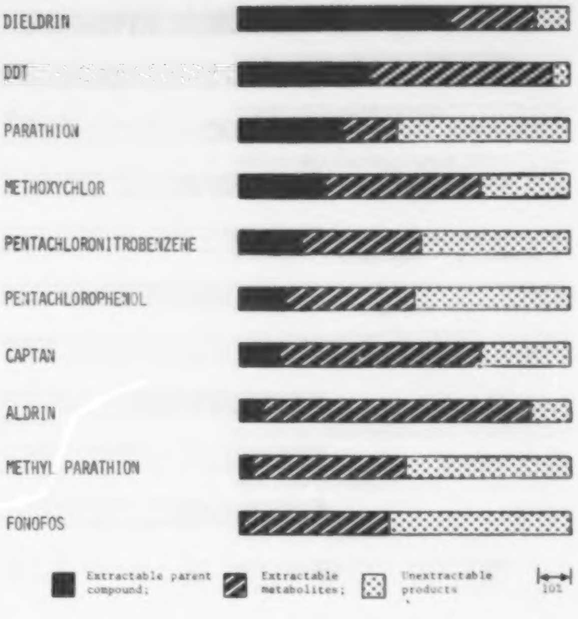


Figure 53. Mean proportions (%) of extractable parent compound, extractable metabolites, and unextractable products detected in the total ¹⁴C-pesticidal residues in terrestrial animals as averaged over 5 species (earthworms, slugs, pillbugs, caterpillars, and voles) from terrestrial model ecosystems; only the dieldrin residue was predominately parent compound. (Degradation within each species and the nature of the metabolites is described in the text.)

From Figures 52 and 53 one would predict that DDT would be accumulated by the animal mass more so than methoxychlor, and that parathion would be more accumulated than methyl parathion. But on the basis of accumulation alone (Figure 52), it would not be safe to predict that DDT would accumulate more than parathion, because the results for DDT were from a vermiculite-containing system and on this basis alone, more DDT should have accumulated in the animal mass than parathion. However, if you also consider the degrees of degradation (Figure 53), it would be safe to predict that DDT would accumulate more so than parathion over a long period of time, because DDT was the more resistant to degradation. In Figures 54 and 55 the same types of comparisons were made, but with a single animal species. The important thing to note is that the relationship among the compounds observed in a single species differed from that of the "average species," as one might expect. It is also important to note in Figures 53 and 55 that there was no sharp demarcation between "biodegradable" and "nonbiodegradable" compounds. Not only can you plot and study a single species as in Figure 54, but it is often advantageous in studying higher animals to make the same type of plot for a single animal part, such as the carcass or some specific organ; the reason should be obvious from Figure 56.

From Figure 54 it might appear that PCNB and PCP would be just as likely, if not more so, to pose a threat to voles as aldrin. But if one examines where within the animal these compounds were located (Figure 56), the previous opinion might change; 68%, 66%, and 10% of the total PCNB, PCP, and aldrin residues, respectively, were located in the gastrointestinal tracts of the various voles. If you reduce the "entire vole" pesticide concentrations by the

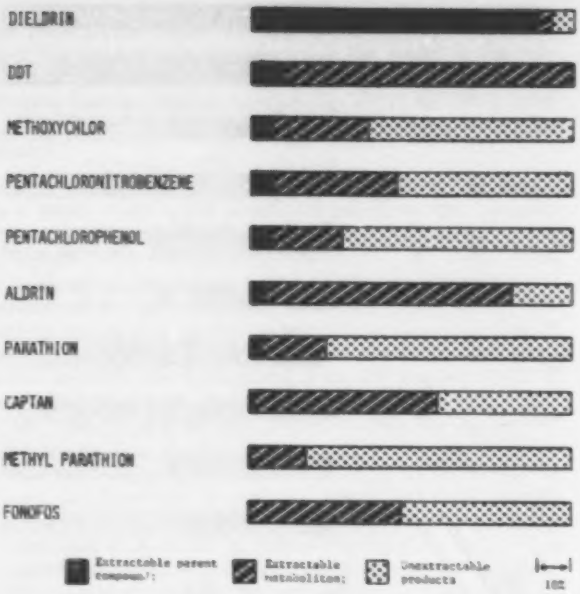


Figure 54. Proportions (%) of extractable parent compound, extractable metabolites, and unextractable products detected in the total ^{14}C-pesticidal residues in the entire vole body. (Degradation within individual vole organs and the nature of the metabolites is described in the text.)

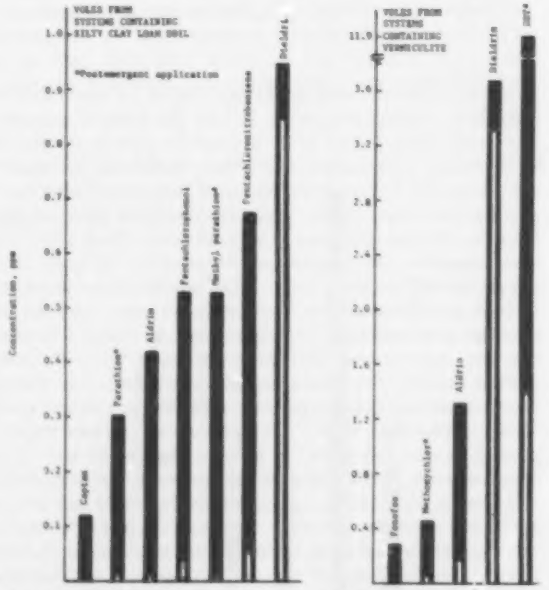


Figure 55. Accumulations of total ^{14}C-pesticidal residues (black bars) and parent compounds (white bars) in voles (entire body) from model ecosystems containing either a vermiculite substrate or a silty clay loam soil. The voles were analyzed after a 5-day exposure within a given model ecosystem. Each system was dosed with a single pesticide at a rate simulating one lb AI/acre; the mode of application was either preemergent to substrate or postemergent to foliage, as indicated. No corn germinated in the soil system treated with dieldrin; the system was drenched with water instead of normal sprinkling.

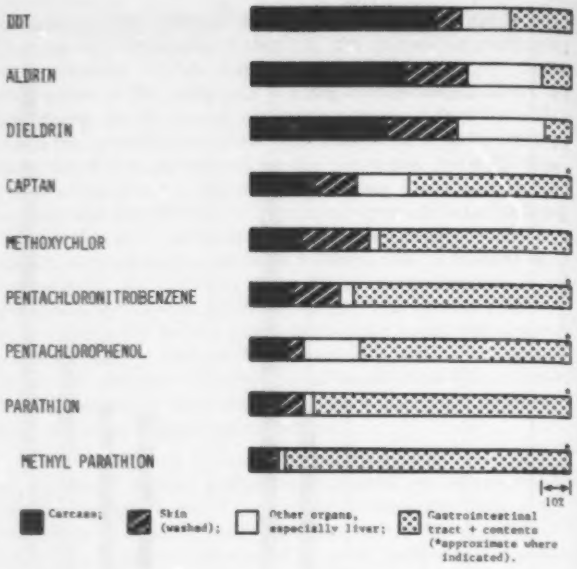


Figure 56. Proportions (%) of the total ^{14}C-pesticidal residue (μg) detected in the entire vole located in various organs. These are distributions of actual quantities, not concentrations. The carcass is the body minus its viscera and skin; it consists predominately of muscle and bone. (Detailed distributions are described in the text and are compared with the relative masses of the organs.

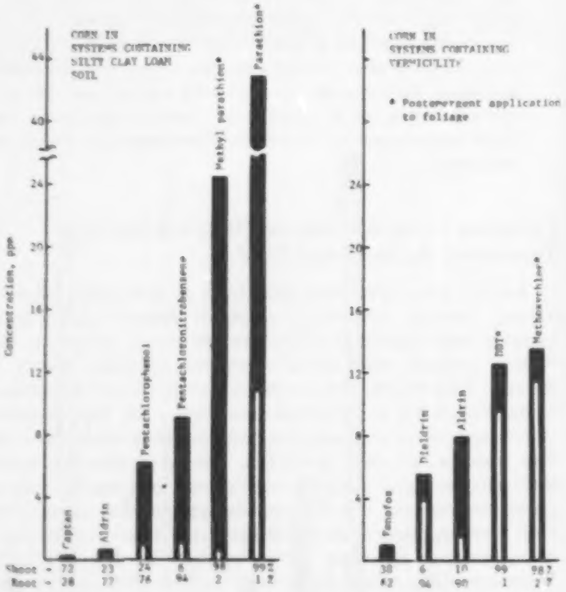


Figure 57. Concentrations of total ^{14}C-pesticidal residues (black bars) and parent compounds (white bars) in entire corn plants 14 days postplanting, and the proportions (%) of the residues (μg, not concentration) located in shoots and roots. Postemergent applications directly to corn foliage were made 10 days postplanting, and preemergent applications to soil were conducted at the time of planting. Each system was dosed with a single pesticide at a rate simulating one lb AI/acre.

above percentages, you will find that the concentration of total aldrin residue in the soma is roughly two-fold greater than that of either PCNB or PCP. (Experimentally this was done in the reverse order; the entire body concentration was found by summing the parts.) Also recall (Figure 51) that fish and snails were much more capable of accumulating PCNB and PCP than dieldrin. Furthermore, in Figure 46, recall that 34% and 51%, respectively, of the PCNB and PCP applications were lost to the atmosphere, whereas virtually none of the aldrin was lost. In Table 15, note that PCNB and PCP were more degradable than dieldrin. Thus, PCNB and PCP are less of a potential hazard than aldrin or dieldrin.

The accumulation (or in the cases where the pesticide was applied directly to the corn, the persistence) and degradation of pesticides in corn are shown in Figures 57 and 58. Figure 57 depicts the concentration in the entire plant; the proportions of this that were located in the root and shoot are indicated at the bottom of each bar. The plants grown in vermiculite treated with aldrin accumulated more total aldrin residue than those plants grown in soil treated with aldrin, indicating that the type of soil definitely affected the quantity of pesticide accumulated by plants.

Hopefully the details as to how this particular laboratory quantitatively and qualitatively concludes that different pesticides affect more than pests differently can be combined with the efforts of others in our desires to better understand and utilize pesticides productively.

ACKNOWLEDGEMENTS

The skillful assistance and unending efforts of Susanne G. Wood, Mary L. Milbrath and Douglas J. Mandel are gratefully acknowledged and deeply appreciated. This study was supported by the U.S. Environmental Protection Agency, grant no. R-80-3249; the enthusiastic guidance of James W. Gillett, the project officer, is sincerely appreciated.

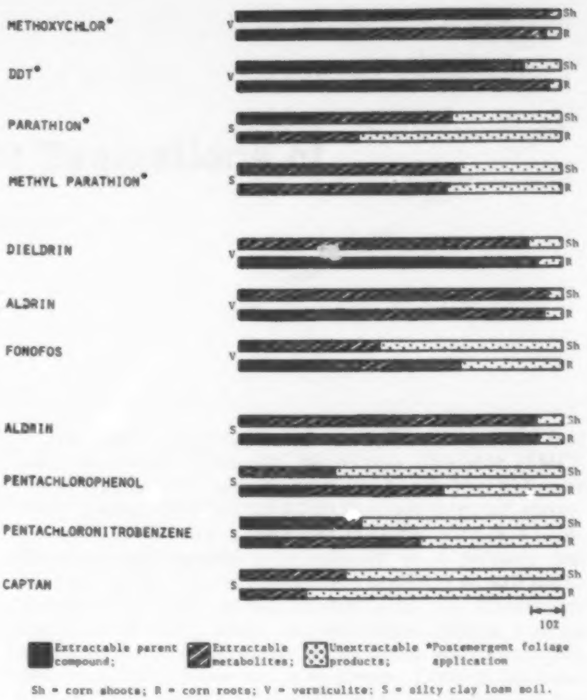


Figure 58. Proportions (%) of extractable parent compound, extractable metabolites, and unextractable products detected in the total ^{14}C-pesticidal residues from corn shoots and roots. Plants dosed by postemergent application were analyzed 4 days after dosing, and plants from systems treated with a preemergent soil application were analyzed 14 days after dosing.

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Terrestrial Microcosm Chamber Evaluations of Substitute Chemicals

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ABSTRACT

The disposition of ^{14}C-dieldrin (HEOD) was examined in triplicate in a Terrestrial Microcosm Chamber (TMC) model ecosystem consisting of a synthetic soil medium, agricultural crops, numerous invertebrates and a microtine rodent. This was the first series of experiments testing the application of the TMC to evaluation of substitute pesticides. Residues were determined in air, soil, water, plants, invertebrates, and tissues of the vole (Microtus canicaudus) and her offspring. Design considerations for this experiment were (a) whether the test system satisfactorily measured the fate of the material applied, (b) whether the results were comparable to field and laboratory studies of the rates and extent of movement, transformation and biological effects of HEOD, and (c) whether the TMC was comparable to other microcosm test systems. The average accountability was 89% of ^{14}C-dieldrin applied to the 3 TMCs. Dieldrin adversely affected both vole survival and reproductive performance

Subsequently modifications of the TMC have been applied to evaluate the fate and effects of parathion, methyl parathion, hexachlorobenzene, pentachloronitrobenzene, pentachlorophenol, p-nitro-phenol, captan, 2-4,5-T, simazine, bromacil, and trifluralin. Valuable information in regard to problems associated with the physical and biological components and with respect to future research needs have developed out of these tests.

I. DIELDRIN (HEOD) DISPOSITION

Introduction

Recognition of the paucity of information on the behavior and potential effects of chemicals accidentally or purposefully dispersed into the environment has created a new awareness of problems associated with the introduction of new chemicals as substitutes for pesticides found to be "environmentally difficult." In attempts to screen adverse environmental behavior, attention has turned to the use of controlled laboratory model ecosystems or microcosms.

Metcalf et al. (1971) and Cole et al. (1976) have successfully tested aquatic-terrestrial and terrestrial monoculture systems, respectively, with a variety of pesticides. More recently, Beall et al. (1976) have reported construction of a larger model agroecosystem tested with toxaphene and DDT (Nash, et al., 1977). In general, these model ecosystems use controlled environmental parameters (light, temperature, moisture, and air and water flows) and defined biotic and abiotic components to produce a laboratory system representing certain general processes characteristic of agroecosystems.

This report concerns the behavior of dieldrin in the first full tests of the Terrestrial Microcosm Chamber (TMC), which was detailed elsewhere (Gillett and Gile, 1975 and 1976). Dieldrin [HEOD; 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*endo, exo*-1,4:5,8-dimethanonaphthalene] was selected as a primary test material, because its environmental behavior is well documented, it is only poorly metabolized, its effects are described on many target and non-target species, and its widespread use might require replacement with substitutes about which much less would be known. Dieldrin has been studied in the aquatic-terrestrial system (Sanborn and Yu, 1973) and in the terrestrial monoculture system (Cole et al., 1976). As will be seen, the results in the TMC are highly consistent with the behavior of the chemical in the natural environment and in other microcosm systems.

METHODS AND MATERIALS

Microcosm Chamber

A detailed description of the TMC construction is available (Gillett and Gile, 1975). Briefly, the TMC consists of a glass box (1 m × 0.75 m × 0.61 m) with a Plexiglas® lid containing ports for sampling and for the air and rain systems. Pairs of TMCs rest on plywood frames, providing a 2% grade, under banks of fluorescent and incandescent lamps which deliver about 2500 ft-candles at the soil surface at the peak of the 16-hr daily photoperiod. Temperatures within the TMC range from 30°C during the day to 18°C at night.

Membrane- and charcoal-filtered air enters the TMC at 10ℓ/min and exits under vacuum through a cellulose prefilter and a 5 μ-Metricel® filter at the same flow rate. Exhausted air passes through a series of water-jacketed and refrigerated (-15°C) condensers and then through a series of gas scrubbing bottles containing NaOH solution (Figure 59). Before release to the environment, the air is passed through NaOH-impregnated, activated charcoal filters.

Reverse-osmosis purified water may enter the TMC through either the "rain" or "spring" systems. The "rain" system consists of an array of four nozzles each containing a perforated ceramic disc capable of spraying a 38 x 50 cm surface. The "spring" system, which utilizes the same water source, consists of a 6-mm ID polypropylene tube extending 1 cm above the soil surface. Delivery is regulated by an in-line flowmeter.

Water is removed by a micropump via a groundwater sampling tube positioned in one of the low end corners of the TMC. The groundwater is filtered through the same cellulose and Metricel® filters as the air samples, then enters a 10-ℓ reservoir where it can be conveniently sampled (Figure 59).

Soil

The bottom 5 cm of the TMC consists of a layer of pea-sized river gravel and then a layer of washed, coarse river sand (8 grit), about 40 kg each. The remaining 20 cm of soil is composed of a mixture (by weight) of 10% Jiffy Mix Plus™ (organic matter), 45% 20-grit sea sand, and 45% Turface®, a heat-expanded illite clay. The soil temperature is monitored via thermistor probes placed throughout the upper layers.

Flora and Fauna

Alfalfa (*Meticago sativa*) and perennial ryegrass (*Lolium perenne*) were selected after numerous trials on the basis of the most satisfactory growth within the terraria and for their ability to support the voles and invertebrates. Moreover, they represent the broad leaf plants and grasses, respectively, and both are of economic importance.

Two bacteriaphagic nematodes, *Pristionchus iheriteiri* and *Cephalobus perseghis*, are included as consumers of bacteria elicited by plant exudates and animal wastes as well as serving as food for other invertebrates. Earthworms (*Lumbricus* spp.) provide another important link between soil-bound pesticides and other biota while effecting physical distribution of the pesticide through the soil as a vertical grazer. Laboratory-reared Enchaetriad worms are also being studied, since this latter annelid will tolerate a broader range of temperatures.

Two pillbugs (*Armadillarium* and *Porcellia* spp.) represent the arthropod detritivores. The adults are not generally subject to predation; however, inadequate food supply can lead to cannibalism. Juvenile pillbugs appear subject to both cannibalism and predation, thus providing a pathway between decaying plant matter and higher organisms.

Third and fourth instar larvae of mealworms (*Tenebrio molitar*) are added primarily as food for the vole, but their scavenging and subterranean habits expose them to soil-bound pesticides and can thus effect the bio-transfer of these chemicals or their metabolites.

Achetus domesticus, the common brown house cricket, is an omnivorous orthopteran which serves primarily as a food source for the vole. Their habits provide a ready pathway for transfer of pesticides, while their reproduction in the soil may serve as a sensitive index of toxicity.

Although the adult garden snail, *Helix pomata*, is not preyed upon by other inhabitants of the TMC, their young are prey for the voles. The adult snails are a terminal repository of soil- and plant-borne residues, reproduction is carried out in the soil, and the adults provide some competition for the vole as herbivores. Snails represent the order Mollusca and, along with slugs, constitute a major pest of many crops and ornamentals.

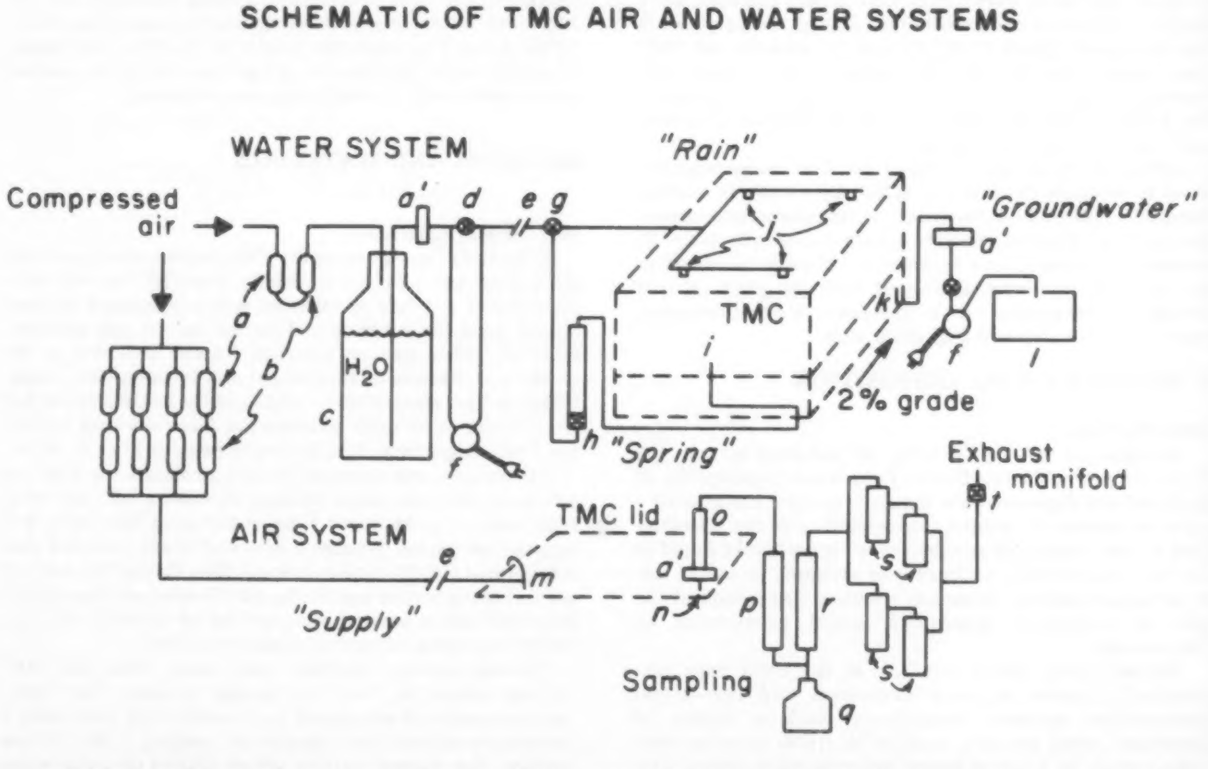

Figure 59. Schematic diagram of air and water systems a. 5 μ filter capusle (a'. 47 mm 5 μ Metricel filter); b. charcoal filter capusle; c. 25-1. carboy; d. by-pass valve; e. equivalent parallel plumbing to each TMC; f. Micropump™ ; g. three-way valve for "rain" and/or "spring" supply; h. needle-valve controlled flowmeter; i. "spring" inlet tube; j. "rain" nozzles; k. "groundwater" outlet tube; l. 10-1. water sample reservoir; m. air supply inlet; n. air sampling system outlet; o. flowmeter; p. 400-mm Graham-type condenser (water-cooled); q. 1-1. pyrex condensate receiver; r. 400-mm Allihn-type condenser (refrigerated to −15°C); s. gas washing bottles with coarse filter and 300-ml solvent; and t. exhaust vacuum regulating valve.

Microtus canicaudus, the gray-tailed vole, is indigenous in the ryegrass and alfalfa fields of the Willamette Valley. These voles, which have an 18- to 21-day gestation period, mate well in captivity and produce litters of 4 to 7 young with weaning occurring between 15 to 20 days postpartum. Such reproductive behavior facilitates a variety of possible tests and is indicative of the potential pest problem sometimes evidenced by the microtine rodents in agroecosystems. The intense appetite of the vole along with its omnivorous nature permit it to consume the entire biotic contents of the terrarium (except for microbiota and adult snails), thus suiting it well for biomagnification studies.

Radiolabeled Material

The ^{14}C-dieldrin used in this test was a technical grade (at least 85% HEOD) supplied by the Radiochemical Centre (Amersham, England). The label is in positions 1, 2, 3, 4 and 10. Radiochemical and chemical purity were confirmed by TLC and gas chromatographic analysis.

Reagents and Chemicals

All solvents were scintillation grade or pesticide grade, depending upon the use, with the exception of reagent grade ethanol and acetone used to clean the TMCs at termination. Unlabeled HEOD was an analytical standard from City Chemical Co. Other chemicals were reagent grade. (Note: the use of trade names of reagents and materials is intended solely for the reader's benefit and does not impute endorsement of that product to the exclusion of others serving equally well. The Environmental Protection Agency makes no such endorsement or warranty of performance).

EXPERIMENTAL PROCEDURE

The soil mixture was added to the TMC in three 8-cm deep layers (approx. 40 kg each) along with 6ℓ of water. Each new layer was compressed overnight with a weighted plywood sheet before the next layer was added. During this process the light cycle was initiated and the room and the operating TMC temperature established. The earthworms and nematodes were added with the last layer.

Within 24 hours of adding the worms the TMC was planted with 30 g of ryegrass seed scattered on one longitudinal half of the surface and 20 g of alfalfa seed ("Thor" var.) on the other half. The seeds were covered with 1-2 cm of soil mixture and a fine layer of sea sand, which reduced algal growth during sprouting. Ten liters (1.3 cm) of water were added through the "rain" system at planting and every other day for the first week. More water was added later when the TMC appeared desiccated (soil moisture probes included with the thermistors having failed after the first week).

On day 10 after planting, with the seeds well sprouted and plants growing well, 50 pillbugs, 50 *Tenebrio* larvae, 6 adult snails, and crickets were added. In a test of the impact of cricket loading on the growth of the young plants, no crickets were added to Terrarium VI, 50 crickets were added to TMC V, 100 crickets to TMC VII, and 200 to TMC VIII. Cricket censuses and plant counts and dry weight yields of samples were followed for 19 days.

On day 40, TMCs V, VII, and VIII were treated with ^{14}C-dieldrin applied in acetone (4 ml) at the rate of 1.116 pounds/acre (1.38 kg/ha, 50 μci of ^{14}C-dieldrin diluted with reference standard grade HEOD from City Chemical Co. for a total of 103.5 mg of HEOD/TMC). The ^{14}C-dieldrin was applied via an aerosol sprayer to the plant foliage. TMC VI was treated only with an equal volume of acetone, thus serving as the control.

Additional *Tenebrio* larvae were introduced on days 42 and 53 and crickets were added to all operating TMCs on days 47, 53, 55, 58, 59, 60, and 62. Ten g of mixed alfalfa and ryegrass seeds were also added as food on days 55 and 60.

A gravid female vole was added to each terrarium on day 48 approximately 18 days since her last litter (and presuming effective conception during postpartum estrus, about 18 days through gestation). The "spring" system was activated at a rate of 1 ml/min. It was intended that the vole be maintained for 3 weeks or until all vegetation was gone, but when the initial voles died, TMC VIII was terminated and new gravid female voles added to TMCs VII and V. When those animals died, TMC VII was terminated and a female vole who had whelped the night before was added to TMC V until it was terminated at her death on day 67. The initial control vole survived throughout the duration of the test.

Daily censuses of visible animal species were conducted, while weekly soil cores were taken for nematode enumeration. Samples were taken weekly for ^{14}C analysis in plants and crickets. Air filters were sampled on a daily basis, as were the scrubber contents and condensate for the first three days. Subsequently the latter two were sampled every third day. The groundwater was collected periodically and sampled at termination.

When a TMC was terminated, the macrobiota were removed, enumerated, and stored at -20°C until analysis. The soil was divided into zones consisting of a 10-cm edge band (which provided "corner" samples at the intersections) and a remaining center section which was sampled diagonally in 5 rectangles approx. 10 x 15 cm. The vertical soil profile was divided into layers approximately 6 cm deep. Soil samples were stored at -20°C until analyzed. When all of the soil had been removed, all interior surfaces were scraped clean of snail feces, etc., and the TMC and remaining gravel washed exhaustively with acetone, ethanol, and finally water until free of radioactivity.

Analysis

All analysis for ^{14}C was conducted using a Packard Model 3385 Tri-Carb liquid scintillation spectrometer. Automatic external standardization was used to correct for quenching down to an efficiency of about 40% below which the sample was corrected using an internal standard (^{14}C-toluene). Except where noted, most samples were counted in a toluene/Permafluor® cocktail. Aqueous samples were counted in Aquasol®.

The condensate and groundwater were extracted with ether after initial counting of an aliquot, and the ether was counted. The NaOH solution from the scrubbers was treated with $Ba(OH)_2$ solution and the resulting precipitate and solution extracted with ether, which was counted. The $BaCO_3$ precipitate was suspended in the gel phase of toluene/Permafluor® and counted.

A 100-g subsample of each soil sample was extracted in a blender with 4 ml/g of a hexane: isopropanol (3:2) mixture. If an aqueous phase separated after filtration, it was removed and counted. The organic phase was counted, as was the acetone used to re-extract the residual soil material. Generally, little radioactivity was detected in the acetone re-extraction. The residue was dried and weighed, thus providing a basis for all calculations. Four 1-g subsamples of the soil residue were combusted in a Packard Model 206 Oxidizer and counted in Carbo-Sorb®/Permafluor® cocktail. One sample was spiked with ^{14}C-decalin as a correction for the procedure (combustion, separation, and counting).

The plants were divided into root and shoot portions, the former having been gently washed and blotted dry to remove the majority of soil particles. Each portion was homogenized separately in 2 ml of acetone per g along with 1 g of Na_2SO_4 (previously washed with hexane and dried). After filtration, the extract was counted and the residue was resuspended in 6N HCℓ solution. The suspension was warmed overnight at 60°C and then filtered and the filtrate extracted with ethyl ether. The aqueous and ether phases were both counted, and the residue from filtration was oxidized to determine "bound ^{14}C."

Animal tissues were dissected, if possible, and then treated by essentially the same scheme as for soil samples, except that 1 g of Na_2SO_4 was added during homogenization (to prevent development of an aqueous phase). "Bound residues" were determined by use of the Oxidizer.

If an organic extract of soil, plant, or animal tissue contained in excess of 10^4 dpm, the sample was further analyzed by TLC on silica gel G plates (500 μ) developed by hexane:ether (1:1). If necessary, the sample was reduced in volume on a rotary evaporator (with 1 ml of 2,2,4-trimethylpentane added as keeper). Materials on the TLC plates were located by radioautography, removed manually and counted. Since recoveries of standards were 95% of that applied, values were uncorrected. The relative proportions of the recovered materials were used to determine the percent composition of the extracts, thus permitting conversion of radiochemical values to ppm dieldrin or equivalents of dieldrin as metabolites. For a few heavily quenched samples, the ^{14}C content of extracts was corrected on the basis of ^{14}C recovered from TLC plates. No aqueous sample showed more than 10^3 dpm of unextractable ^{14}C, so these were not investigated further.

RESULTS

One of the major goals of microcosm experimentation is to provide a rapid and simple method, in lieu of full field tests, to assess the environmental fate of a candidate chemical. In this experiment, the first consideration is whether the test system provided a satisfactory measurement of the fate of the mass of the material applied. Secondly, did the test system provide reasonable comparability to other field data and laboratory tests, especially alternative microcosm systems? Thirdly, are the rates and extent of movement, transfer, and transformation consistent with other experience? Finally, were the effects observed consistent with the known activities and described fate?

Mass Balance

As shown in Table 16, the average recovery of ^{14}C was 85.7% for the three test systems. When estimates of known losses are considered, the average accountability is 88.6%. Consideration of error terms in the individual determinations of radiologic and chemical analyses suggests that absolute values are ±30% for composite samples (soil, plants) due to spacial variations. Replicated determinations were usually in the range of ±5% for soil samples. Additional unaccounted losses include those initially missed in spray monitoring, where some aerosol may have left the chamber. Swipes of the outside of the lid of TMC VII were approximately three times higher than TMCs V and VIII. Generally speaking, however, accountability in the TMCs greatly exceeds that of field studies (40-60%) and approaches that for test tube and whole animal trials (90-100%).

The three TMCs were not direct replicates, inasmuch as they were operated for different times, and apparently differences in performance resulted from variations in the control systems. Considerable difficulty was encountered in maintaining even air flows through the TMCs. Water would condense in the flow meters and filters, and measured air flows in all terraria dropped to below 1ℓ /min for much of the experiment. The most inconsistent observation was that of water balance, wherein only 25% of input could be assessed as output through groundwater, biota removed, condensate recovered, and water vapor passed through. However, small errors in measurement of initial soil water level could have provided substantial error to through-put calculations, since terminal water content (43 kg) greatly exceeded through-put (17-19ℓ). Re-evaluation of potential leaks showed that some air might enter at points other than the air inlet, but that neither water nor air could leave elsewhere.

Table 16—^{14}C Mass Balance (10^6 dpm)

Item	Terrarium VIII (12 days)	Terrarium VII (20 days)	Terrarium V (27 days)	
Sprayer content	111.00	111.00	111.00	
Spray losses	2.23	6.53	1.87	
Applied to TMC	108.77	104.47	109.13	
Soil	78.13	43.66	44.32	
Plants	14.34	24.91	49.96	
Air	0.84	1.04	0.27	
Water	nd	nd	0.03	
Fauna				
Voles	1.32	3.34	2.34	
Snails	1.30	1.16	1.78	
Invertebrates	0.59	0.85	0.49	
Other[a]	4.44	1.04	0.82	
Total recovered	100.97	76.00	100.01	
Loss estimates:				
Voles[b]	0.12	0.49	0.25	
Plants[b]	—	2.68	0.22	
Soils[b]	2.0	—	—	
Nematode[c]	0.08	0.05	0.04	
Air[d]	0.82	1.23	0.26	
Total estimated	3.72	4.45	0.77	Mean:
Percent recovered	92.8	72.7	91.6	85.7 [0.132][e]
Percent accounted for	96.2	77.0	92.3	88.5 [0.115][e]

[a] Includes nesting materials, vole and snail feces, algal swipes from walls, and terminal clean-up of TMC.

[b] Samples lost or spilled, est. from equivalent samples.

[c] Based on est. soil residues.

[d] Based on est. of scrubber-trapped material after first 4 days, prior to which scrubber samples were handled improperly.

[e] Coefficient of variation in brackets.

[f] nd = less than 10^3 dpm/1.

Residue Patterns

a. Soil. The bulk of the residual activity, although initially applied to the foliage, was found in the soil. The majority occurred as free HEOD and none of the principal metabolites (9-OH-dieldrin, photodieldrin, 9-ketodieldrin or the dehydrohalogenated fused ring compounds similar to photodieldrin) were detected at the 1% level by TLC. Figure 60 shows the distribution of HEOD, metabolites, and bound residue within the principal sectors (edge and corners; center) by level for each of the TMCs. Metabolites were essentially origin materials; bound residues were combustible

^{14}C derived from extracted (hexane/isopropanol; acetone) soils. In preliminary tests treatment of soil residues with 6N HCℓ at 60°C overnight failed to release ^{14}C. Unexpectedly high proportions of metabolite/HEOD and bound residues/HEOD occurred in TMC VIII; moreover there was substantial movement into lower layers of soil at the edges and corners. The lowest layer was inadvertently discarded prior to analysis of TMC VIII. The vertical profiles in TMCs VII and V are consistent with a compound of low leachability and low rate of degradation and for which diffusion and mechanical movement (digging by earthworms or vole) may have played the principal distributive roles.

b. Plants. A cursory examination of Table 16 would suggest that plants are accumulating residues of ^{14}C from labeled dieldrin. However, the temporal residue data (Table

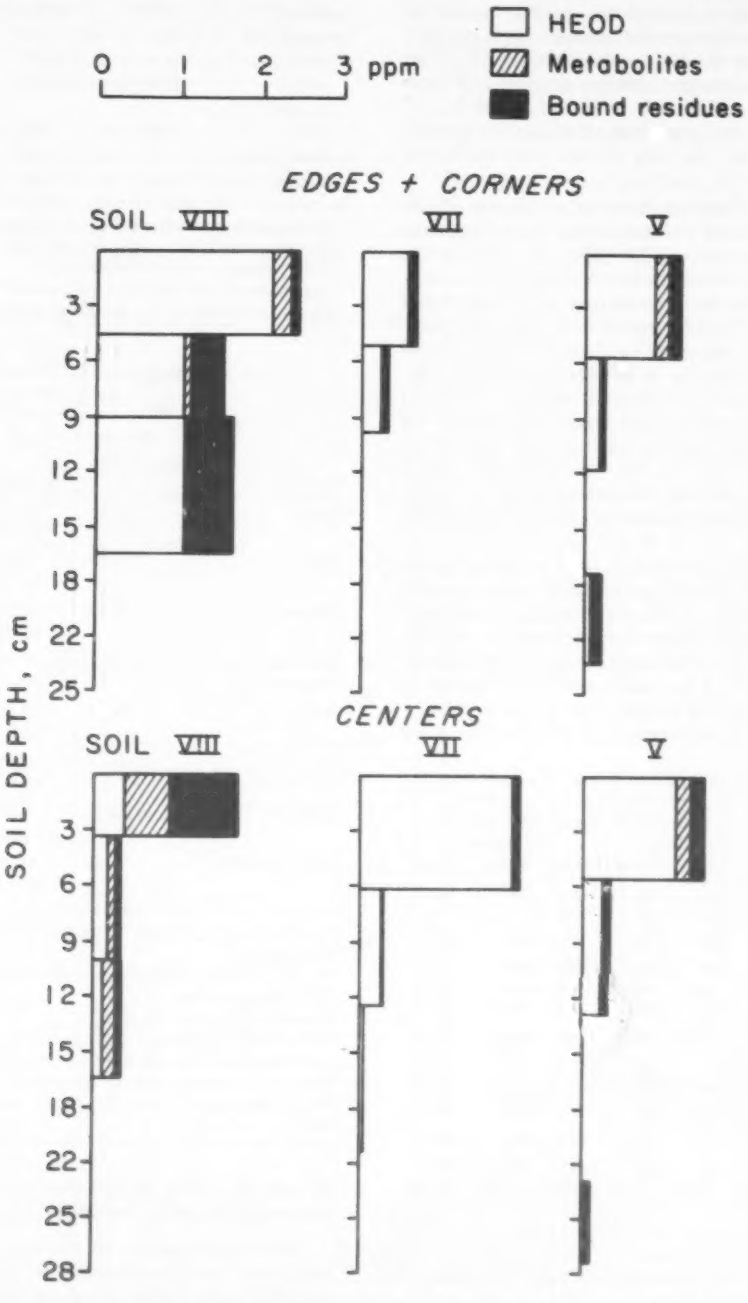

Figure 60. Distribution of HEOD, metabolites, and bound residue in soil mixture. Soil depth of layers was determined from the sum of the dry residual masses of the several layers and dividing the depth proportionately. Residue values on ordinate represent means of 10 analyses for "edges and corners" and 5 analyses for "centers" in layer I, 7 and 5 analyses for layer II, and 2 analyses in each sector of both layers III and IV. The areas represent the masses of HEOD or equivalents in the respective sectors and layers.

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17) reveal that declines in HEOD and total ^{14}C proceeded at relatively comparable rates from different initial loadings of the rye and alfalfa. Metabolite and bound residue values were low for all plant tissues. Small sample sizes hindered accurate determination of root contents, but it is apparent from soil data that translocation of dieldrin need not be considered to account for the high root residues. Initial levels would be expected to be about 20 ppm (if 3 kg of plant material intercepted about 2/3 of the spray). Desiccation of samples between harvest and weighing (during removal of soil, washing and dissection) might account for both high variability and exceptionally high residue values. The first order decay rates of whole plant residues suggest half-lives of 3.1, 5.0, and 12.3 days for dieldrin in alfalfa in TMCs VIII, VII, and V, respectively. Ryegrass had a half-life for dieldrin of 9.1 days in TMC VIII, the only system with sufficient data for judgment.

c. Invertebrates. Patterns of residues of HEOD (Table 18), metabolites, and bound residues were most consistent with time and between terraria for pillbugs, adult snails, and snail larvae. The earthworms had low and more variable contamination, while the more omnivorous crickets and *Tenebrio* tended to be slightly higher and less consistent. Among the fauna, snails are second only to the voles in total activity of which the majority is maintained without apparent metabolism (Figure 61). Bound residues exceeded HEOD only in *Tenebrio* adults and larve from TMCs VII and VIII, but were an appreciable part of total activity, in crickets (19%), earthworms (11.1%), and pillbugs (8.3%). Metabolite patterns in crickets resembled those of voles, whereas snail TLC patterns resembled that of the starting material.

d. Voles. Two of the voles were in such advanced states of decomposition when found that tissue or organ residue analyses were impossible. Otherwise highly consistent findings of organ and whole body residues characterize this test species (Table 19). In the absence of significant adipose tissue deposits, the bulk of the residue was distributed as might be expected, except for a high level in the reproductive organs. It remains to be determined whether these are exceptionally rich in lipids or have specific binding factors materially affecting residue accumulation.

Table 17—Dieldrin residues (as HEOD) in alfalfa and ryegrass

Days since treatment	TMC	ppm HEOD (fresh weight) Alfalfa Root	Alfalfa Shoot	Alfalfa Total[a]	Ryegrass Root	Ryegrass Shoot	Ryegrass Total[a]
2	VIII	146	303	300	48.2	92	88.4
	VII	556	269	280	19.6	*	*
	V	205	462	449	*	220	220[b]
9	VIII	125	104	104	48.5	119	70.3
	VII	243	72.4	73.4	54.0	68	66.3
	V	208	248	247	14.1	83.4	39.1
12	VIII	17.0	9.0	27.9[c]	20.6	14.6	39.7[d]
					24.1	47.2	39.1
15	VII	20.9	46.7	45.1	511	63.7	81.4
	V	188	272	268	*	142	142[b]
27	V	18.0	102.3	100.6	93.7	130	122[c]
Mean[e]		173 (0.92)	189 (0.76)	190 (0.73)	116 (1.48)	98 (0.59)	89 (0.66)

[a]Weight-averaged mean of root + shoot.
[b]Since foliar biomass greatly exceeded root mass, the value for shoot was taken for the whole plant.
[c]Includes an additional ether extract.
[d]Does not include value for seeds, 19.7 ppm.
[e]Coefficient of variation in parentheses (s.e./$\bar{x}$)
*Lost sample.

Table 18—HEOD residues in macroinvertebrates from TMCs treated with ^{14}C-dieldrin by foliar spray (ppm HEOD fresh weight[a])

TMC Species	VIII	VII	V	Mean[b]
Earthworm	3.6; 1.0	4.4	5.2	3.2[0.62]
Snails,				
adult	25.0(9) 21.3	28.5(9) 15.3	23.7(12) 45.8(15); 29.0	26.9[0.35]
juveniles	128	102	84.6	104.8[0.21]
Tenebrio,				
adult	5.1	5.7	37.3	18.4[1.15]
larvae	0.71	1.6	4.3	2.2[0.85]
Crickets,				
live	4.8(9); 3.6	17.7	2.1	7.1[1.02]
dead	—	5.5	2.8	6.4[0.91][c]
Pillbugs	21.7	33.9	25.5	27.0[0.23]

[a]Numbers in parentheses indicate samples taken other than at termination which was day 12 for VIII, day 20 for VII, and day 27 for V, after treatment with ^{14}C-dieldrin.
[b]Numbers in brackets are coefficients of variance (s.e./$\sqrt{x}$).
[c]Mean for combined live and dead crickets.

Table 19—Residues of HEOD and Metabolites in Voles[a] (ppm fresh wt.)

Tissue	Percent body wt.	HEOD	Metabolites[b]	Bound Residues
Brain	1.03 (4)	17.67 [0.14]	0.45 [0.60]	0.19 [1.71]
Liver	7.11 (4)	35.57 [0.29]	0.93 [0.34]	0.98 [1.19]
Kidney	1.69 (3)	27.93 [0.26]	0.89 [0.36]	0.44 [1.12]
Reproductive organs[c]	2.19 (2)	77.06 [0.79]	nd —	1.43 [1.41]
Heart and lung	2.04 (3)	14.09 [0.20]	1.13 [0.52]	0.39 [1.10]
Stomach and g.i. tract[d]	22.46 (4)	22.19 [0.52]	1.46 [0.56]	0.93 [0.86]
Carcass[e]	64.40 (4)	28.81 [0.48]	1.31 [0.88]	0.24 [1.10]
Whole body[f]	(6)	26.16 [0.40]	1.28 [0.52]	0.34 [0.94]

[a]Coefficient of variation in brackets, numbers of organs analyzed in parentheses.
[b]Includes both polar (origin) and non-polar materials detected by TLC (ether:hexane::1:1).
[c]Includes uterus and ovaries.
[d]Includes contents of g.i. tract, pancreas, spleen, and mesentary.
[e]Includes skin, hair, skeleton, and musculature.
[f]Does not include pups or fetuses, for which HEOD = 7.09 (12) [0.22], metabolites = 0.39 (11) [0.38], and bound residues = 0.13 (12) [0.77].

Most significantly, all the pups (neonatent or *in utero*) had lower concentrations than the dams and showed considerable homogenity of values. No trend in relation to position *in utero* was evident.

e. Air, Water, and Other Media. Only a fraction of a percent of the total activity was recovered in the air (Table 16) and only the water sample from TMC V had detectable activity, as might be expected from a compound as water-insoluble as dieldrin. The majority of activity recovered from the air was on the filters, but the proportion trapped in the liquid scrubbers increased considerably as time progressed. The total daily activity (Figure 62) trapped in the air was very erratic, but semi-log plots against time show that the slope of the air residue decay curve parallels that of the residue loss curve for alfalfa. Part of the erratic nature of these plots may be reflections of altered activity patterns within the terraria, as when voles are added or die. Nash et al (1977) found similar results in examining loss rates of DDT and toxaphene in an agroecosystem.

Indices of Performance

Metcalf et al. (1971) has utilized two conceptual indices of pesticide disposition: Ecological Magnification (EM) index, the ratio of the residue concentration in a biological element to the concentration in the substrate; and Biodegradation Index (BI), the ratio of polar metabolites (usually characterized as origin material on TLC plates) to parent compound in any biotic or abiotic element. However, the validity and relevance of the reference substrate in the terrestrial system is more open to question than water as the reference in the aquatic system. Because soil becomes the repository of the bulk of residues even after foliar application (Table 16), that substrate concentration was taken as the basis for EM calculations (Table 20). The omnivorous dietary habits of the vole and ubiquity and heterogeniety of exposure (inhalation, dermal, dietary) appear to be more consistently integrated on the basis of soil as reference than on the basis of other components (e.g., alfalfa residues, Table 17), where the coefficients of variation of EM are much greater for both snails and the voles (both depending primarily on the plants as food).

The calculation of BI (Table 21) is not similarly affected, but varies as expected considerably with the species examined. Due to the relatively minor role that transformation to water-soluble metabolites appears to play in overall

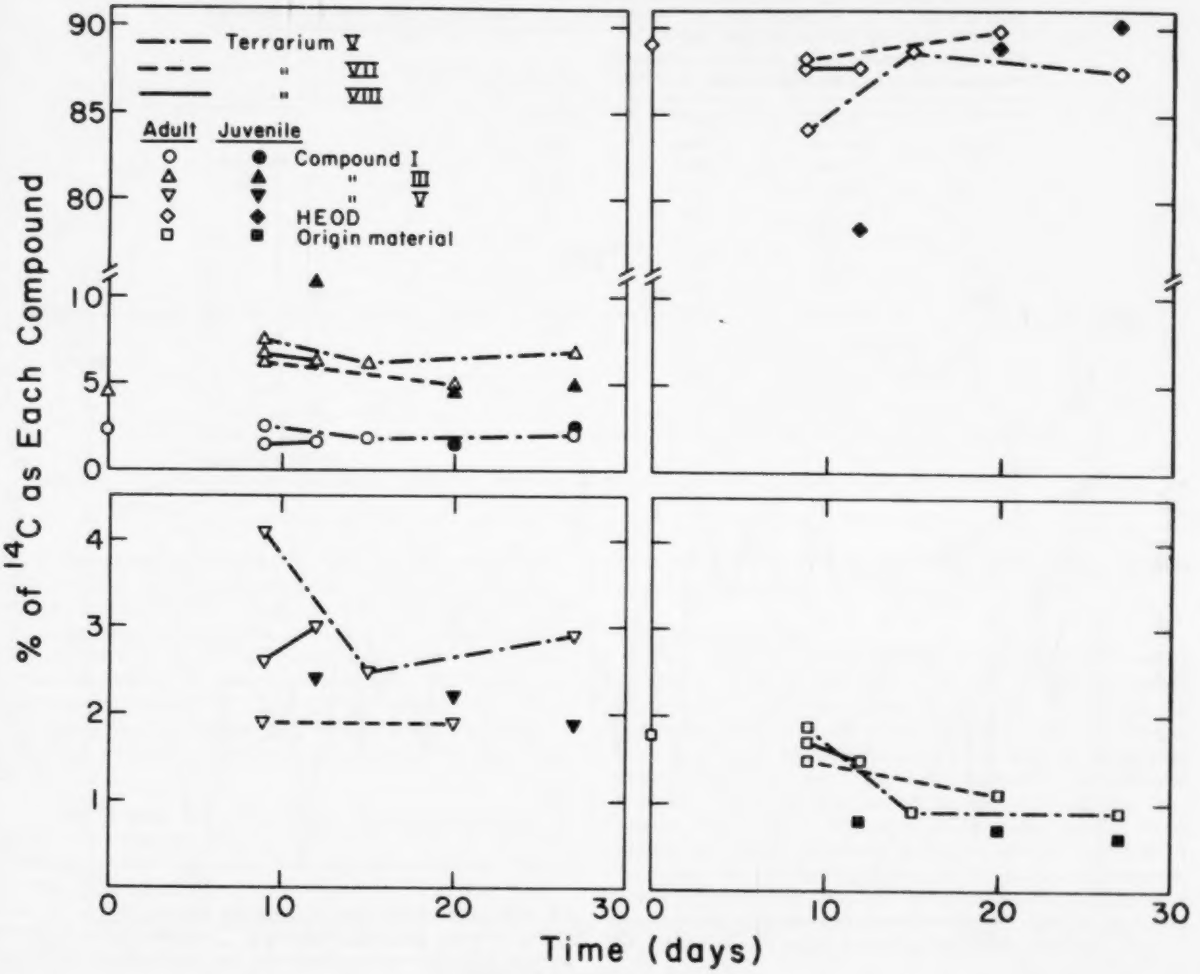

Figure 61. Changes of HEOD, metabolites, and origin material from TLC analysis of snail residues. The materials were identified by R_D in comparison to unrefined ^{14}C-dieldrin: HEOD = 1.0; Compound I = 0.88 to 0.91; Compound III = 0.55 to 0.62; Compound V = 0.44 to 0.48; Origin material = 0.05 (developed on silica gel G, 500 μ, using ethyl ether:hexane::1:1, v/v).

Table 20—Ecological Magnification Index[a]

Species		VIII	VII	V	Mean[b]
Vole female	a	37.8	103.6	56.6	59.5 [0.36]
	b		51.8	50.5	
	c			56.4	
+ pups	a	32.9	78.9		53.8 [0.28]
	b		47.7		
pups/dam	a	0.27	0.15		0.20 [0.31]
	b		0.17		
Snails, juvenile		182.9	226.7	228.6	212.7 [0.12]
adult		35.7 (9)	63.3 (9)	64.1 (9)	61.4 [0.54]
		30.4	34.0	123.8 (15)	
Tenebrio, larvae		1.01[c]	3.56	11.6	
adult		7.27	12.7	100.8	40.3 [1.3]
Pillbugs		31.0	75.3	68.9	58.4 [0.41]
Earthworms		5.14, 1.49	9.78	14.1	7.1 [0.89]
Crickets, alive		6.86 (9)	39.3	5.67	11.9 [1.03]
dead		5.14	12.2, 6.6	7.57	

[a] (HEOD) whole animal/(HEOD) soil at termination; number in parentheses indicate day post-treatment at which ratio was determined. Soil residues and day terminated were: VIII-0.700 ppm, day 12; VII-0.450 ppm, day 20; V-0.370 ppm day 27.

[b] Coefficient of variation (s.e./x) shown in brackets.

[c] Acetone extract only (remainder lost).

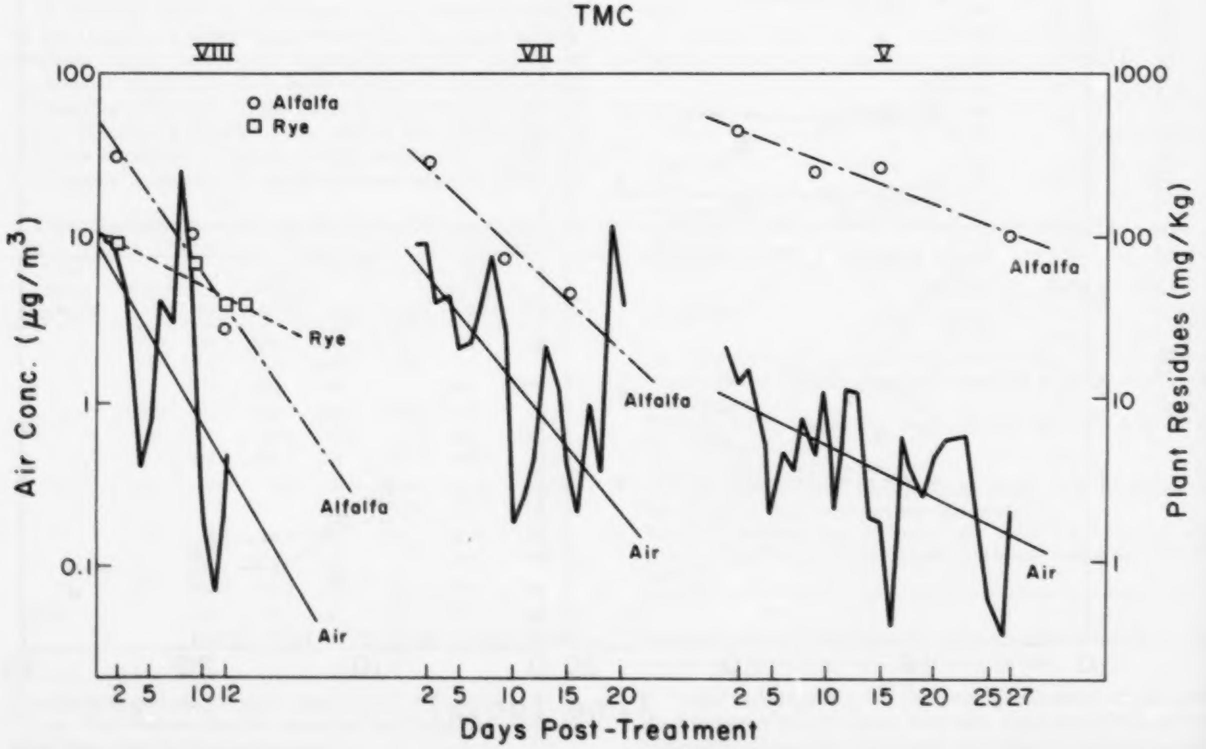

Figure 62. Comparison of daily ^{14}C activity in the air to alfalfa and rye residues. Solid lines connect daily total radioactivity recovered from filters, scrubbers, and condensate. Light solid lines are least mean square estimates of rate of decline in daily output. Broken (alfala) and dashed (rye) lines are least mean square estimates of residue decline on crop.

Table 21—Indices of Toxicity and Disposition in the Vole

Item	VIII	VIIa	VIIb	Va	Vb	Vc	Mean
Body wgt. g.	56.6	66.6	45.6	41.0	36.5	32.5	46.5
Day introduced	9	9	15	9	15	20	
Exposure hrs	35.5	58	71.5	49.5	105.5	142.5	
Brain HEOD ppm	14.9	17.7	*	21.2	17.5	*	17.7
Whole body HEOD ppm	23.3	35.7	23.3	17.6	18.4	20.9	26.2
EM[a]	37.8	103.6	51.8	56.6	50.5	56.4	59.5 [0.38]
BI[b]	0.026	0.017	0.007	0.008	0.005	0.006	0.011 [0.73]
BRI[c]	0.031	0.030	0.113	0.043	0.117	0.091	0.071 [0.57]
TDI[d]	0.057	0.047	0.120	0.051	0.123	0.098	0.082 [0.42]

[a] EM = (HEOD$_{\text{whole body}}$/(HEOD)$_{\text{soil}}$ at termination

[b] BI = (Metabolites)$_{\text{whole body}}$/(HEOD)$_{\text{whole body}}$

[c] BRI = ^{14}C combusted in residue/^{14}C equiv. of HEOD, whole body

[d] TDI = Total (metabolites + bound residues)/$^{\text{(HEOD)}}$ whole body

*Badly decomposed

Table 22—Comparison of Dieldrin Toxicity in Voles

Terrarium vole	Day introduced[d] t_i	Time last seen (hrs) (a)	Time found dead (hrs) (b)	Mean survival time or exposure $\frac{a+b}{2} = t^*$	Day of death $t_i^* = t_i + t^*$
VIII	9	21	50	35.5	10.5
VII a	9	22	142	58[e]	12.4
VII b	15	26	117	71.5	18.0
VI	9	336	—	—	—
V a	9	5	142	49.5[e]	11.1
V b	15	91	120	105.5	19.4
V c	20	124	161	142.5	25.9

[e] Based on previous experience, 48 hrs was subtracted from [(a) + (b)] if no activity was noticed during that entire period.

[d] From day of dieldrin treatment; terrarium VI sprayed only with acetone; others received 10^3 mg HEOD

HEOD disposition, the impact of trace cogeners and breakdown products in the initial pesticide treatment is relatively more obfuscating than it might be for other agents more readily degraded. Whereas the snail (Figure 61) shows small temporal shifts in ^{14}C-labeled components, these same components are drastically reduced in plants and not evident in the invertebrates and the voles. It is not possible to correct for degradation of cogeners to water-soluble compounds (identified only as ^{14}C-labeled origin material) between species.

As can be seen for voles (Table 21), biodegradation of HEOD is limited or very slow, whereas there is a much greater proportion of bound residues accumulating. These residues (characterized solely as ^{14}C-containing) may represent (a) intact parent compound not extractable due to physical entrapment, (b) covalently bound parent compound or metabolites yet possessing a biologically active structure, or (c) assimilated ^{14}C derived from the degradation of parent compound to simple biologically utilizable compounds (e.g., CO_2). Where BI is low and the ratio of BI to BRI (Bound Residue Index, the ratio of bound ^{14}C to ^{14}C equivalents of parent compound in the sample) is large, the problem of potential hazard arising from covalent reaction or secondary toxicity may have to be considered.

Biological Impact

The intense toxicity of dieldrin to the gray-tailed vole is shown in Table 22 and Figure 63. The longer the period after dieldrin treatment before the vole was introduced, the longer the vole lived. By extrapolation we would have had to wait 42 days before introducing the vole to the treated system (at 1 lb/ac equiv.) to have survival comparable to controls. Observation of convulsions in two animals, the failure of exposed pregnant animals to bear and/or wean pups (Table 23), and marked decline in effective activity by the voles are qualitative aspects of the toxicologic impact. The consistent brain residues and body burdens achieved in the animals that died (Table 21) suggest that the repeated mortality of test voles is a valid measure of impact.

DISCUSSION

TMC System Performance

With an average accountability of approximately 89%, the mass balance of ^{14}C-labeled pesticide in the TMC

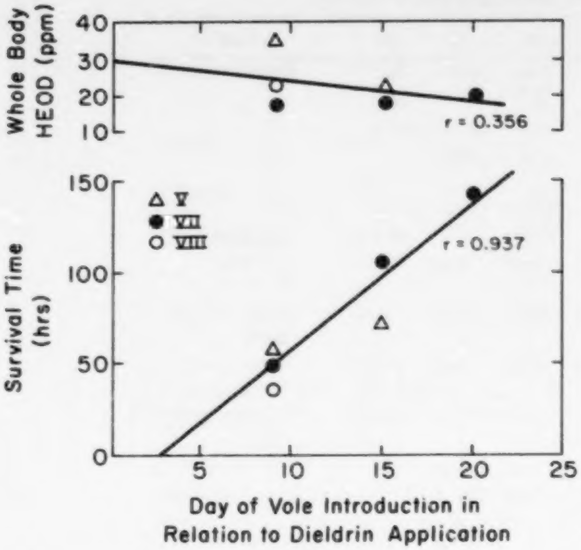

Figure 63. Vole Survival and residues in relation to time of introduction after dieldrin treatment of TMC. The solid lines represent least mean square estimates of correlation.

Table 23—Effects of Dieldrin on Vole Reproduction

Reproduction datum	Experimental (5)[a]	Control (2)[a]
nests	4	1[b]
births	3	2
pups/mating	≥3.2	≥4.0
pups in utero	13	0
live pups recovered	0	0
live pups/mating	0	≥4.0
pups weaned/mating	0	5[b]

[a] number of female voles in parentheses

[b] one birth (3 pups) occurred outside microcosm with female subsequently separated from pups

system is acceptable for preliminary considerations of pesticide fate. The prime difficulty in operation and monitoring concerned the air system, which tended to be erratic. Difficulties arose in estimating pesticide application rate and in assessing distribution in the soil system. Microheterogeneities in transport processes and physical structure of the plant/soil system appear to play a greater role in the more intensive arrangement of the TMC. The alternative to numerous small samples on a spacial basis would be replicate samples of homogeneously mixed total soil (or soil level), as used by Metcalf et al. (1971) or Cole et al. (1976), and should be adequate at the level of resolution provided by the overall disposition. As the mechanisms of disposition are elucidated (through closer spacial and temporal examination of the pesticide distribution), one would anticipate an even lower level of sampling frequency would suffice to describe fate.

Decreased numbers of all invertebrates except snails could not be attributed to dieldrin exposure, inasmuch as similar numbers were recovered from the control terrarium. Snail larvae were about equally distributed by numbers and weight in all terraria, and only one adult snail died. Nematode distributions were not apparently affected by dieldrin treatment, but were associated with rapid growth of the plant species. No phytotoxic effects were noted following an initial "burn" by the acetone solvent. By virtue of its toxicity to the herbivorous voles, the dieldrin treatment protected plan productivity, usually providing the first clue that the vole had died.

As noted in preliminary trials, the voles adapted well to a properly constructed TMC, i.e., one with appropriate food, necessary water, and other conditions to their liking, by building a nest with several entrances/exists and by developing extensive underground runs. Poorly constructed or stressed terraria (e.g., dieldrin-treated) are characterized by small nest with relatively little access and by a higher proportion of superficial runs in comparison to subterranean tunnels. The nests in TMCs, V, VII, and VIII had an average of 1.4 accesses, while TMC VI had 5. Moreover, the nest in VI was twice as large and the tri-level tunnel system was estimated to be over 5 times the volume of the average of the other TMCs.

Prior to dieldrin treatment an attempt was made to estimate the impact of cricket loading on plant productivity. Sampling variation on ryegrass and alfalfa exceeded variation effected by 50, 100, and 200 cricket/box, but counts of cricket numbers visible in censuses from outside the TMC suggested that population numbers followed a first order decline (i.e., $\ln N_{obs}$ vs. t is a linear, negatively-sloped function). Subsequent experimentation has confirmed this and suggests that the slope is affected by toxicant stress, introduction of the vole, and, most radically, by the final extirpation of vegetation. Due to scavenging by *Tenebrio* and pillbugs, dead crickets are not found in numbers corresponding to the rate of population decline.

System Comparability

Results within the TMCs compare reasonably well to those reported for field and laboratory studies, with but few exceptions. Cole et al. (1976) compared foliar application of DDT and methoxychlor to soil application of aldrin, dieldrin, and dyfonate. In the first instance, the vole died with a body burden of 11.9 ppm DDT (EM = 43.4). Aldrin/dieldrin did not result in death, and body burdens were 3.81 ppm (EM = 13.9). Brain dieldrin residues were only 0.57 ppm fresh weight. Thus, route of exposure (via foliage in the TMC test) is critical in comparisons. Irrespective of initial exposure, soil becomes the repository of residues. As was seen in Figure 62, this may be mediated through the air, but also involves processing of plant material to fecal residues and litter. Cole, et al. (1976) found air values of 0.5 to 1.3 $\mu g/m^3$ of aldrin/dieldrin in their system.

Consistency with Past Experience with Dieldrin

The rates and extent of movement, transfer, and transformation in the TMC are generally in agreement with other observations. However, if all of the unaccounted losses were to air, the observed mass lost to air (5-29% in 3 weeks) exceeds the estimated annual field loss of dieldrin to air (4.5%) reported by Parmele et al. (1972). Reported volatilization rates ranging from 0.004 kg/ha/day (Caro et al., 1971) to 3.0 kg/ha/day (Phillips, 1971) encompass the values of 0.60, 1.75 and 0.44 kg/ha/day observed for TMCs VIII, VII, and V, respectively. At 30°C and with an air flow of 300 ml/min Spencer and Cliath (1969) noted a vaporization rate of 0.35 ng/cm²/min when dieldrin was applied at 100 ppm. Field diffusion rates are equivalent to 10-15 air changes per hour, while the TMCs were operated at 2 air changes/hr. Equivalent expressions of rates for the TMCs would be 0.086, 0.25 and 0.063 ng/cm²/min mean losses for TMCs VIII, VII and V respectively. Literature values are very dependent on site (plants, soil), moisture level, temperature, and air movement in a manner not yet precisely related (Spencer, 1976). Similarly, the relative immobility of dieldrin within the soil profile is consistent with a variety of leaching studies (Farmer, 1976), although the properties of the mixture used in the TMC are not those of a true soil.

No effort was made to identify HEOD metabolites and congeners as such, since the organo-soluble metabolites were not present in sufficient quantities. The absence of detectable photodieldrin suggests that photochemical reactions in the TMC are not well reproduced in comparison to field activity. In general, the low rate of metabolism is consistent with various plant and animal studies.

The absence of quantitative data on the effects of HEOD on the test species prevents direct comparison, although interference with rodent reproduction by dieldrin has been observed at levels as low as 0.5 ppm in the diet (Harr et al., 1970). Residues in pups/fetuses were approximately one-third of the whole body concentration of the dam, in good agreement with the work of Hathaway et al. (1967) for rabbits. In the most relevant field study (Korschgen, 1970), soil aldrin/dieldrin residues of 0.31 ppm were associated with dieldrin residues of 0.23 ppm in crickets, 1.49 ppm in earthworms, and 0.98 ppm in *Perimyscus*, whereas the TMCs showed average values of 0.6 ppm in soil, 6.7 ppm in crickets, 3.2 ppm in earthworms and 26.2 ppm in voles.

Conclusions

The TMC system proved quite acceptable with respect to mass balance, although continued improvement in the air analysis is required. Temporal analysis of residues established the rates of flux and the relationship of pesticide flux to biologic performance, e.g., vole toxic response. The major problems encountered (high rate of sampling, large amounts of data requiring reduction) should be responsive to systems analysis and statistical treatment. The TMC represented field experience generally in regard to the major pathways of transformation transport and transfer, but inadequately represented photochemical breakdown.

ACKNOWLEDGEMENTS

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A Microagroecosystem to Monitor the Environmental Fate of Pesticides

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ABSTRACT

A laboratory agroecosystem chamber or microagroecosystem, which simulates field conditions, was developed to eliminate difficulties and expense of following the rates and modes of pesticide disappearance under field conditions. The microagroecosystem is relatively inexpensive to construct (ca. $2,000 each), simply operated, and nearly maintenance free. The microagroecosystem permits simultaneous monitoring of pesticides in four environmental phases; soil, plant, water, and air. Three experiments have been conducted in the microagroecosystems; 1) the insecticides, toxaphene (chlorinated camphene containing 67-68% chlorine) and DDT [1.1.1-trichloro-2.2-bis p-chlorophenyl) ethane]—the fungicides, maneb and zineb [Mn- and Zn-ethylene bis (dithiocarbamate)]; and 3) the herbicides, silvex [2-(2,4,5-trichlorophenoxy) propionic acid)] and 2,4-D[(2,4-dichlorophenoxy) acetic acid]. Results from these experiments indicated that the microagroecosystem may be used in research to obtain preliminary information on the environmental fate of pesticides in the field.

INTRODUCTION

Measuring the disappearance of pesticides under field conditions is often difficult and expensive. Under field conditions, pesticide distribution may be uneven (because of directed spray or granular application and drift) and affected by runoff, leaching, volatilization, and atypical seasonal conditions and fluctuations. Sampling and monitoring of the environmental factors are expensive and time consuming, and usually only 1 experiment/year can be established.

Because of the ease and lower cost of collecting samples and accumulating data on the environmental fates of pesticides in laboratory ecosystems, as compared with collecting such information under field conditions, the laboratory ecosystem approach has recently gained popularity. However, until we can use the results from laboratory ecosystems to predict the fate of pesticides under field conditions, we must verify these results under field conditions.

Environmental chambers are not new. Ward (1852) used glazed glass chambers during the 1830's to prevent London smog from killing sensitive plants and to ship plants by sea. Its first uses were to exclude adverse environmental factors rather than to simulate a specific environment. Subsequently, other growth chambers were developed, primarily to establish a specific environment and are available commercially. These are capable of simulating almost any type of plant environment. However, growth chambers are expensive and designed for pot experiments, and they do not fit our concept of a microcosm, or more specifically a microfield situation. Hence, to meet this need we designed and developed a microagroecosystem.

Southwood and May (1970) compared an agroecosystem (microagroecosystem) with a natural ecosystem (microcosm): 1) They are not self-perpetuating; 2) They are limited in duration, ranging from a few months for annual to several years for perennial crops; 3) They contain plants selected by man (rather than the end product of natural selection in a given environment); 4) They contain monocultures maintained by cultivation and modern herbicides, (except intercropping, mixed orchards, and forage-crop mixtures); 5) They are limited in the range of genotypes with maximum synchronization of flowering and maturation; 6) They are fertilized with chemicals or manure with concomitant gains in rate of growth and nutrient levels in foliage; 7) Their soil moisture may be controlled by irrigation and drainage, and 8) They have periodic pest outbreaks.

DESIGN AND CONSTRUCTION OF MICROAGROECOSYSTEM

The microagroecosystem is an enclosed glass chamber located in a greenhouse. It is maintained near ambient conditions by pulling a large volume of air through the chamber with a suction fan (Figure 64). It was designed for studying the environmental fate of a pesticide under field situations.

A partial design of the microagroecosystem has been published (Beall, Nash, and Kearney, 1976; Nash, Beall, and Harris, 1977). The system includes a glass chamber and table; chamber exhaust manifold; air suction fan and table; suction-fan-exhaust manifold; sprinkler system; lighting; electrical power; moisture, temperature relative humidity, and air velocity sensors; and optional automatic data acquisition equipment for moisture, temperature, relative humidity, and air velocity measurements.

Chamber Subsystem Design

Chamber

Each chamber was constructed from 1-cm (3/8 in) plate glass (Figure 64) (Renwar Scientific Co., Gaitherburg, Md. 20760) and is held together with clear silicone aquarium cement. The chamber's interior measures (150 cm long, 115 cm high, and 50 cm wide) with a total volume of 0.75 m^3, after allowing for a 15-cm soil layer. The all-glass chamber was assembled in a permanent 2-cm (0.75 in) plywood tray lined with 0.6-cm (0.25 in) thick felt padding. Tray wall height is 15 cm above the chamber floor.

One side of the chamber has two sliding service panels (70 cm high by 76 cm long), each of which has two 2.5-cm

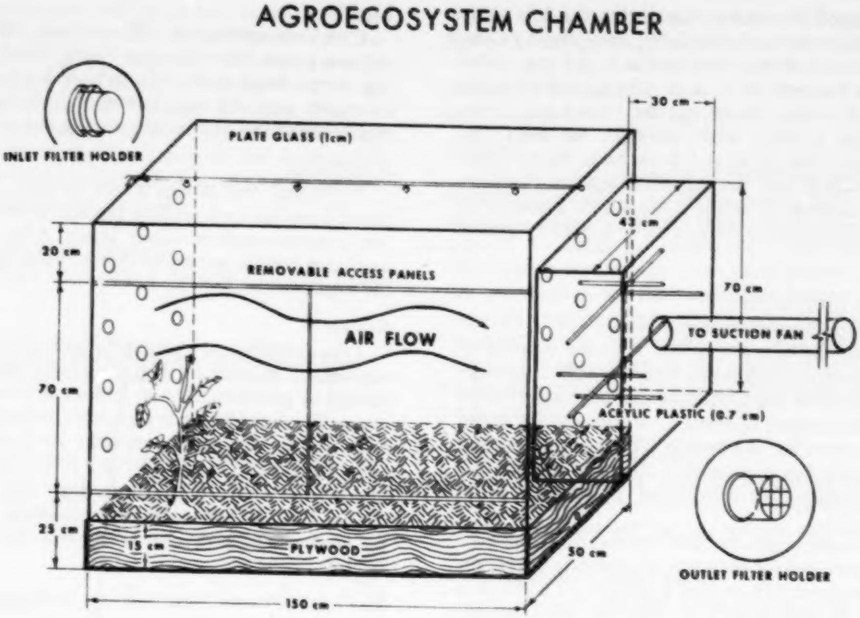

Figure 64. Diagram of microagroecosystem chamber.

finger holes for sliding or lifting. (A No. 6 rubber stopper is placed in each hole during operation.) Each panel slides on an aluminum "H" track 1.25 cm wide (inside) and 1.4 cm deep, secured to the top and bottom chamber walls with aquarium cement, and overlaps the wall edges. Each "H" track is 1 cm thick, therefore, each upper and lower wall piece was cut 1.5 cm shorter in width (18.5 cm top and 23.5 cm bottom) to accommodate the "H" track and felt padding.) The "H" track sides and the end of each chamber adjacent to the panel is felt lined (1 mm thick by 1.25 cm wide) to assure a tight fit. Also, the bottom of the "H" track and one end of an access panel are lined with a strip of polyurethane foam weather-stripping. A 1-cm width piece of teflon tape placed over the weather stripping in the "H" track provides for easy sliding.

Both ends of the chamber contain 12 5-cm holes for air intake and outlet. They are centered 20 cm apart vertically (beginning 35 cm from the chamber bottom) and 16.7 cm apart horizontally. A 2.5-cm hole for accessories is centered 6 cm above the air-intake end and 15 cm below the air-outlet end. Another 2.5-cm hole for drainage is centered in the chamber bottom 15 cm from the air-outlet end.

Supporting table

The supporting table (54 cm wide, and 40 cm high) is constructed from 6-cm angle-iron frame and 3.75 cm-diameter legs, which can be adjusted. From end to end, the tables slopes 1°.

Air Subsystem Design

Trapping filters

Polyurethane foam is used for air-inlet and-outlet filters. The foam (William T. Burnett Co., Baltimore, Md.) is an ester base, open-cell type with a density of ca. 0.032 g/cm³. Air-inlet filters were cut from 0.3-cm thick sheets with a 7-cm pipe similar to a cork-borer sharpened on one end. Air-outlet filters (5 cm di) were cut from 5-cm sheets.

Each air-outlet filter is held in place with 68-mm long glass holder (Figure 64), made from 45 mm i.d. borosilicate glass tube with 2-mm thick walls (Renwar Scientific Co.). The holder intake end is expanded to 46 mm i.d. for 30 mm of its length. The rim has a 3 mm rounded lip to retain a rubber 0-ring for sealing. The outlet end of the holder contains a glass rod grill. The holders, containing the filters, are inserted from the inside of the chamber.

Each air-intake filter is held in place with a holder made from clear 5-cm o.d. acrylic tubing (Figure 64). The holder is 3.5 cm long with a 1 cm wide collar, made from 6.5-cm o.d. acrylic tubing, cemented 1.5 cm from one end. The other end of the holder is milled off ca. 1 mm from the collar so the inlet filter, when placed over the inlet end, is held in place by a second removable collar. The holder fits snuggly when inserted through a 5-cm O-ring attached to the chamber air-inlet hole with industrial adhesive. The air-inlet filters keep out dust and insects and provides a slight (0.2 to 0.5 cm water) negative pressure inside the chamber.

Chamber manifold

An air-outlet chamber manifold encloses all 12 air-outlet filter holes to assure equal suction through each filter (Figure 64). The manifold is constructed from clear acrylic plastic sheet (0.65 cm thick). The inside of the manifold is reinforced with six pieces of extruded acrylic tubing (1.8 cm o.d.) for the 12 to 13 cm water negative pressure in the manifold. The manifold is cemented together by using CH_2Cl_2, which dissolves and welds the acrylic plastic together. The manifold is butted against the air-outlet chamber-end and contains 12 6-cm holes patterned exactly the same as the air-outlet chamber holes. The manifold is attached to the air-outlet chamber end by applying a very thin bead of caulking compound around the manifold edge; then, with a set of air-outlet filters in place, the suction fan is turned on for ca. 30 sec to ensure an air-tight fit.

(Prolonged running of the suction fan during the first 24 hr could create air channels in the caulking compound.) Centered on the other end of the manifold is a 12.7-cm outlet hole onto which a piece of 30.5-cm acrylic tubing (11.5 cm i.d.) is attached. A collar, 18 cm square by 1.5 cm thick, made from acrylic plastic, adds strength between the manifold and suction fan pipe. A 1.3-cm hole, 23 cm from the manifold, is located near the top of the suction-fan pipe for an air-velocity sensor. A second 1.3-cm hole is placed in the center of the manifold top for a relative humidity sensor cable.

Air-suction fan and table

Air is pulled through each chamber with a 115 V, 1/3 HP high-pressure direct-drive suction fan. A mean volume of 2.5 m^3/min (3.3 volume changes/min) can be pulled through the chamber, when both the air-intake and air-outlet filters are in place. Under normal operating conditions, the mean air-velocity in the chamber is 5 m/min. The fan is connected to the plastic suction-fan pipe with a 25-cm length of 12.7 cm i.d. flexible-spring-steel-reenforced nylon and vinyl covered hose.

A wood-frame table supports both the manifold and suction-fan 50 cm apart. (More accurate air-velocity measurements could be obtained if they were 1 m apart). The table is 50 cm wide, 110 cm long, and 75 cm high for the manifold and 85 cm high for the suction fan.

Air-suction-fan-exhaust manifold

For safety, all air pulled through the chamber is exhausted from the greenhouse through a manifold, constructed from duct sheetmetal (27 cm i.d.)—a horizontal piece for five microagroecosystems (4.8 m) and a vertical piece extending ca. 1.5 m above the greenhouse ridge. Each suction fan is connected to the manifold with a piece of flexible hose (described above).

Irrigation Subsystem Design

Sprinkler

A sprinkler system centered 6 cm from the top extends the length of the chamber. A 2.5-cm access hole at the air-intake end allows for access for 0.6 cm i.d. brass tubing. On the opposite end, a 10-ml beaker is cemented (6 cm from top) to the inside of the chamber to hold the sprinkler end. Teflon tape, wrapped around the brass tubing on the air-outlet end, and a rubber stopper on the air-inlet end provides a cushioned, rigid, and air tight sprinkler subsystem.

Each sprinkler has four nozzles (Model 1/8TTGO.3 from Spraying Systems Co., Wheaton, IL) spaced 37.5 cm apart. Each nozzle delivers 160 ml water/min at 1.46 kg/cm^2 pressure to give a solid cone spray (2.5 cm of "rain" in ca. 29 min). Between the tap water source and each chamber is an adjustable pressure regulator and a time-clock-controlled solenoid valve.

Drainage

Drainage is provided and leachate samples are collected from a Teflon-stopcock glass column (12.5 x 1.5 i.d. cm) attached to the chamber bottom, 15 cm from the air-outlet end. The column is held in place with a milled Teflon collar. The column is filled with glass wool, and a stainless steel screen (0.35 mm) placed over the column prevents loss of solid materials. The glass wool must be pushed against the screen to provide a "wick effect" during drainage.

Lighting

One time-controlled, 107 cm long, 180 W low-pressure-sodium-vapor light (Norelco-North American Philips Lighting Corp., Hightown, NJ), which hangs 5 cm above the chamber, provides supplemental lighting for cloudy days and extends the day length during the winter months.

Electrical

Electrical requirements are 20-amp (60 cycle, 110 V) fused lines for each suction fan and a 600-V source for each low-pressure-sodium-vapor lamp. Also, a line should be available for the sprinkler system solenoid and other possible accessories.

Space

Five chambers are presently located in a greenhouse and require a 7 m wide by 4 m deep floor space. Each chamber should be positioned 1.1 m (center to center) apart for servicing. The greenhouse is temperature controlled with steam heat during the cooler months and cooled with water-evaporative pads and suction fans during the warmer months. Relative humidity is not controlled. All external connections to the microagroecosystem are located above the chamber for convenient and safe movement around the system.

Environmental Monitoring Subsystem

One primary objective in designing the microagroecosystems was to develop a system that was simple to operate; hence, to monitor the existing environmental factors (i.e., the greenhouse), rather than to control the environmental factors with elaborate and expensive equipment.

Temperature

Three points in the chamber are monitored for temperature: 1) the soil, (ca. 5 cm deep in the chamber); 2) near the air-outlet end, (level with the lower filters); and 3) near the air-inlet end, (level with the upper filters). Each temperature sensor is made from thermocouple wire.

Relative humidity

A five-stage, wide-range, relative-humidity sensor (Model 15-1818, Amer. Inst. Co., 8030 Georgia Ave., Silver Spring, Md., 20910) is installed in the air-outlet manifold box near its center.

Soil moisture

A soil-moisture sensor (15 cm) (Model 2725, Soil Moisture Equipment Co., P. O. Box 30025, Santa Barbara, CA 93105) is located at mid-point near the panel-access doors of the chamber.

Air flow

Air flow through the chamber is measured with a velocity sensor. A hot-wire anemometer (Model AB-27 Hastings Air-meter., Hastings-Raydist, Hampton, VA) is inserted through a hole in the rigid plastic pipe (See air-outlet manifold above) between the air-outlet manifold and suction fan.

Automatic Environmental Data Acquisition (Optional)

Several environmental factors can be measured periodically with relatively inexpensive equipment or with automatic equipment. Automatic data acquisition equipment (Leeds and Northrop, North Wales, PA, 19454) automatically records temperatures and relative humidity periodically. Soil moisture varies slowly, except after an irrigation, and, therefore, is only read once daily. Soil moisture can be maintained within a given range, inexpensively, with soil moisture switching tensiometers. Presently, we are investigating sensors for automatically recording air velocities from a point-source, but they are not economically feasible for our present method since 10 measurements are necessary. Possibly, we can find a point within the air-outlet manifold or between the suction fan and air-exhaust manifold, which will accurately represent movement of air through the chamber.

Estimated Construction Costs

An estimate of the construction costs is listed in Table 24.

Table 24—Estimated Microagroecosystem Construction Costs

Item	Item Cost	Sub-system Cost
1. Chamber subsystem		$ 850
Chamber (completely assembled holes in glass, felt, plywood base, and delivered)	$750	
Supporting table	100	
2. Air subsystem		720
Trapping filter holders		
Inlet (25 @ $10.00)	250	
Outlet (12 @ $5.00)	60	
Chamber manifold	150	
Air-suction fan	80	
Chamber manifold and air-suction fan table	80	
Air-suction-fan-exhaust manifold ($400 for 5 systems)	80	
Polyurethane foam filters (60 filters/experiment)	20	
3. Irrigation subsystem		60
Sprinkler	40	
Drainage	20	
4. Lighting		50
5. Electrical		200
6. Space		—
7. Assembly and installation		200
Total		2,080
8. Environmental monitoring subsystem		1,260
Temperature (thermocouple)	50	
Relative humidity + cable	340	
(Hygrotran transmitter (Model L-15-6376)—capable of serving several R.H. sensors, (Amer. Inst. Co.)	400	
Soil moisture	20	
Air flow meter and sensor (capable of serving several systems)	450	
9. Automatic environmental data acquisition		13,000

OPERATION AND MAINTENANCE

Experiment Initiation

The felt, foam weather-stripping, and Teflon tape around the chamber access panels are replaced for each experiment. A quantity of glass wool is placed in the drainage column and a screen placed over the drainage hole.

Five sets of air-filters should be preextracted before initiation of the experimentation.

Soil

The soil we have used in our experiments is a Galestown sandy loam with a pH value of 6.7, organic matter content of 5.2%, and 0.33 bar moisture tension at 15.6% water content. We use this soil because of its accessability on the Agriculture Research Station at Beltsville, Maryland.

The soil is sieved through a 6 mm screen before it is placed in the chamber to a 15 cm depth. Each chamber requires ca. 0.12 m^3 (165 kg) of soil.

Plants

The microagroecosystems were designed to study the environmental fate of pesticides under field situations, thus, the crop or plants selected will vary depending on the objective of a particular experiment. However, experiments should be programmed to use those plants that grow well in the greenhouse for that particular season. Most crops, except corn, can be grown to maturity. For row crops, we germinated the seeds in pots, then selected plants of similar size for transplanting in the chamber after 30 to 50 days. Crops like cotton and tomatoes at 5 plants/chamber is a good crop density.

Pesticide Application

All pesticides are applied, according to present or past (DDT) labeled instructions. To date, our application methods are not optimal, so we are still experimenting.

Emulsifiable Concentrate

We use a hand-held spraying rig with one nozzle (See sprinkler above) to apply emulsifiable concentrate pesticide formulations. The nozzle is attached to a brass elbow, which is attached to a 60-cm (0.6 cm i.d.) brass boom, fitted with a toggle-operated, shut-off valve at the handle end. Teflon tubing connects the sprayer handle and a stainless steel vessel (250- or 1,000-ml sample cyclinder) containing the pesticide solution. Pressure is applied to the vessel with compressed (1.46 kg/cm^2) gas.

Before pesticide application, we remove the two access panels and place a sheet of polyethylene film completely around the inside chamber walls, except for the air-inlet and -outlet holes. We also close the access opening. Then we cut a slit lengthwise (ca. 30 cm above the plants) to insert the spraying apparatus on the access side. The pesticide solution is applied by several uniform sweeps the length of the chamber.

Granular

Presently, granular pesticide formulations are applied by pouring the granules through a 1.5 mm sieve that is hand-shaken ca. 30 cm above the plants.

Radio Labeled Pesticides, Pesticide Degradation Products, or Pesticide Contaminants

The microagroecosystems are especially useful for applying and containing ^{14}C- and ^{3}H-radiolabeled pesticides and hazardous pesticide degradation or contamination products. The chambers have a slight negative pressure (0.2 to 0.5 cm water), which prevents contamination of the surroundings and aids in protecting the worker during sampling. Finally, the air passing through the chamber is filtered. (The polyurethane foam filters are checked in advance for their trapping efficiency for each compound.) However, the filters do not trap CO_2 and H_2O. Consequently, compounds from which large volumes of $^{14}CO_2$ or $^{3}H_2O$ may evolve from the chamber environment should be avoided, unless an adequate exhaust system is provided.

A further advantage of the microagroecosystem over field and pot experiments is that the cost of applying radioactive pesticides, contaminants, or degradation products may be rather nominal as compared with field applications, since these compounds are often very expensive.

Sampling

A sampling schedule for a 35-day period could resemble that given in Table 25.

Table 25—Sampling Schedule for Microagroecosystem

Day	Air	Plant	Soil	Water	Chamber
0	X				
0.1	X	X			
0.4	X				X
1	X	X	X		
3	X	X			X
4			X		
7		X		X	
8	X				
9					X
14		X	X		
15	X				
18					X
21		X			
24	X		X	X	
28		X			
32			X		X
35	X				

Plant

An advantage of the microagroecosystem over a pot experiment is that the abundance of plant foliage provides an opportunity for sampling several times. After pesticide application, plants are sampled immediately (after drying), and then periodically until termination of the experiment. The surface area of the control plant sample is measured, and fresh and dry weight of the plant samples are determined.

Soil

The large volume of soil in the microagroecosystem chamber gives adequate soil for sampling. Normally, soil samples are taken periodically beginning 1 day after pesticide application. This is primarily because the many samples (air and plant) collected on day 0 make further sampling impractical. Initially only the surface 1-cm of soil is sampled (15 2-cm cores). Then, to follow the mobility in the soil of the compound under investigation, periodically 10 (2 cm) cores samples are taken the depth (15 cm) of the soil and separated into 5-cm increments.

Air filters

Before use, the air-outlet filters are Soxhlet extracted for 12 hr with hexane:acetone (1:1 v/v). The filters are further extracted with additional solvents (usually hexane, methanol, water), depending upon the compounds being tested. After drying, the filters are stored in glass jars. Most filters can be reused several times, with only about a 10% loss after each period (because of hardening or creases forming as a result of repeated extraction).

All 12 air filters are collected immediately after application and replaced with clean filters. A second collection is made within 0.5 to 5 hr (depending on the compound under investigation) after application of the pesticide. Usually, three collections besides the treatment collection, are taken within the first 24 hr, then at approximately exponential time periods afterward. Filters collected from each chamber are pooled and considered one sample.

Water leachate

Only leachate water has been sampled so far. Although the microagroecosystem was designed to collect surface runoff samples, the technology for determining runoff under field conditions is superior to that which we could develop. Because of this and the large number of other samples generated by the microagroecosystem, we have not collected surface runoff samples.

Leachate samples are obtained periodically by applying 1 to 3 cm (depending on the existing soil moisture) of water. A few minutes are allowed after sprinkling ceases to assure soil absorption of water that might be between the soil-glass interface. The drainage stopcock is opened and the first 2-liters of leachate are arbitrarily collected. If no drainage occurs, the process is repeated with smaller increments of water, until leachate is obtained from the saturated soil. (The glass-wool in the drainage column must be in contact with the screen and soil to initiate and maintain leaching).

Chamber sides

Since the interior glass surfaces of the chamber are a significant portion of the total surface available for deposition during pesticide application and later during pesticide volatilization, we are presently monitoring the chamber sides for pesticide residues. However, results indicate this may not be necessary in the future.

A series of microscope glass-slides (130) are attached in series to the inside of the chamber (backside, two ends, and top) in rows with a small amount of caulking compound (tub and tile).

Twenty-six slides are collected immediately or within 0.4 day after pesticide application and then periodically afterward. A designated fraction of the 26 slides collected are from each surface to assure adequate and random sampling.

Since we covered the interior walls of the chambers with polyethylene film during pesticide application, any residue that may be found on the slides, therefore, would have resulted from volatilization of the pesticide from soil or plant surfaces.

Any samples (primarily soil and chamber slides), which cannot be extracted immediately, are stored at -5°C.

Analyses

Monitoring the fate of a chemical in the microagroecosystem is limited primarily by capabilities of the analytical unit to analyze the compounds under investigation and by the large number and diverse types of samples. In one recent experiment, over 5,000 individual measurements were conducted on the various samples. Therefore, any analyses of microagroecosystem samples is beyond the scope of this paper.

However, we have had good success in extracting a wide range of compounds from our samples with a standard extraction procedure. All of the air filters and many of the plant and soil samples can be Soxhlet extracted. We use the 40 mm i.d. Soxhlet extraction tubes and now have five banks of six Soxhlets each. Our extracting solvent is hexane:methanol:water (100:35:15). This forms a two-phase mixture, which is capable of extracting both polar and nonpolar compounds. Further, the two-phase extract aids in separation of polar and nonpolar compounds for extract cleanup.

Operation and Maintenance

One of our major objectives in designing and constructing the chamber was to obtain a system that would be simple to operate and require little maintenance. Our microagroecosystem requires little or no attention for several days. After the system has been prepared for operation, usually the only maintenance requirement is plant irrigation about every 10 days. Automatic irrigation equipment will be installed in the near future. (A pest problem would have to be dealt with the same as in field or pot experiments). With two experiments, we had to top the plants (tomato and cotton) because when they reached the top of the chamber, water condensed between the glass and leaf surfaces and in the chamber corners on cool days with very high light intensity. All other aspects of the microagroecosystem operations and maintenance to date have been problem free.

Cleanup and Disposal of Wastes

After each experiment, the soil and all felt sealers around the access panels are removed and placed in plastic containers for disposal, if radioactive compounds are used. The chamber interior is wiped with a soapy cloth, which is disposed of also. Then the chamber is further cleaned with large amounts of soapy and clear water and allowed to drain.

Any potential radioactive material is disposed of through our normal radioactive waste disposal system. Since the volume of soil is not large, filling or removing soil is done with a shovel or hand scoop and wheelbarrow.

APPLICABILITY AND VERSATILITY

Based on our experience, the microagroecosystem will have a wide range of applicability toward determining the environmental fate of agricultural chemicals in simulated field situations and possibly in microcosms. Further, they might be adapted for certain special situations of industrial chemical accidents as well.

To demonstrate their versatility, we conducted three experiments with three different classes of pesticides. The first experiment, cotton, was grown and was sprayed with a toxaphene or DDT emulsifiable concentrate six times at 7 day intervals. The insecticide residues were monitored during the treatment periods and for 56 days afterward (Nash et al., 1977).

The second was with the fungicides, maneb and zineb and their degradation product, ethylenethiourea (ETU). Tomatoes were grown and were sprayed twice with maneb and zineb emulsifiable concentrate. The second application was after 30 days with ^{14}C-labeled fungicides. Residues were monitored throughout the experiment, which lasted 70 days after the second application. Only part of the ^{14}C results will be presented here, because all the data has not been fully summarized.

Our last experiment was with the herbicides, silvex and 2,4-D and the contaminate, TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) in certain silvex samples from the past applied to Kentucky bluegrass turf. While the experiment is still in progress, residues of silvex and 2,4-D were monitored over a 70-day period with applications on days 0 and 35. Silvex was applied both as an emulsifiable concentrate and with a granular fertilizer and 2,4-D was applied with the granular fertilizer.

RESULTS AND DISCUSSION

Residues of all pesticides were found in all four environmental phases; soil, water, plants, and air. Residues in soil, plants, and water seemed to be typical of those under field conditions. Technology for determining residues in air has not been well developed until recent years, consequently, little information is available on pesticide residues in air. However, with the microagroecosystem, we are beginning to accumulate a body of information on the comparative volatilizations of several pesticides from plant and soil surfaces.

Figure 65 shows the accumulation of toxaphene and DDT on a soil surface from repeated applications. The persistence of these compounds is demonstrated by their persistence for nearly 2 months after the last treatment. Their mobility in soil is very low (Table 26).

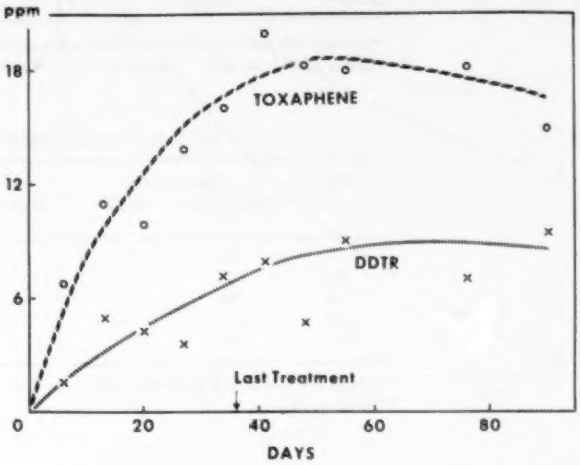

Figure 65. Toxaphene and DDTR residues in surface 1-cm soil of microagroecosystem chambers. DDTR includes p,p'-DDE, -DDD, -DDT, and o,p'-DDT.

Table 26—Insecticide Residues with Depth in Soil (Dry Weight) at End of Experiment

Depth, cm	Compound, ppm			
	DDTR	Toxaphene	Maneb[a]	Zaneb[a]
0-1	9.47	14.9	—	—
0-5	2.49	4.56	0.40	0.27
5-10	0.09	0.48[b]	0.02	0.05
10-15	0.06	0.14[b]	0.02	0.04

[a] Based on ^{14}C. [b] Values probably high, because very low amounts with present method of determination gives high (50%) values.

Also, the mobility of maneb and zineb is very low with most (91 and 75%, respectively) of the residues found in the upper third of the soil profiles.

Pesticide residues on or in plants at the beginning and end of the experiments are summarized in Table 27. DDTR (p,p'DDT, DDD, DDE, and o,p'DDT) and toxaphene are more persistent than silvex and 2,4-D. Several clippings of grass were sampled between the beginning and end of the herbicide (silvex and 2,4-D) experiment, whereas only leaves were taken periodically from the pesticide and fungicide experiments.

Table 28 shows the amounts of pesticide residues in the leachate from the microagroecosystems. We have arbitrarily analyzed the first 2-liters of leachate from a collection. The total amount of leachate, during any single collection, was more than double this amount on occasion because of excess water applied to saturate the soil. Perhaps, a more accurate assessment of any single collection would be periodic grab-subsamples of the total leachate. Greater amounts of the more water-soluble (acids) herbicides

Table 27—Pesticide Concentration on Plants at Beginning and End[a] of Experiments

Compound	Plant	Application kg/ha a.i.	Beginning ppm	Beginning µg/cm²	End ppm	End µg/cm²
Toxaphene	Cotton	2	4,400[b]	11.3	715[b]	1.83
DDT	Cotton	1	1,610[b]	3.40	340[b]	0.87
Maneb[c]	Tomato	2	1,400[b]	2.75	66[b]	0.15
Zineb[c]	Tomato	2	1,420[b]	2.80	91[b]	0.20
Silvex	Bluegrass	2	192	0.253	0.18	0.06×10^{-3}
Silvex[d]	Bluegrass	1.3	4	0.005	0.002	0.06×10^{-6}
2,4-D[d]	Bluegrass	2.7	6	0.008	0.002	0.08×10^{-6}

[a] Concentrations at 90, 70, and 28 days for the insecticides, fungicides, and herbicides, respectively.
[b] Based on dry weight. [c] Based on ^{14}C. [d] Granular formulation.

Table 28—Pesticide Residues (µg/1 litter) in Water Leachate

Day	Insecticide[a]		Fungicide[b]		Herbicide		
	Toxaphene	DDT	Maneb	Zineb	Silvex		2,4-D
					Concentrate	Granular	Granular
7	—	—	—	—	81	31	161
28	25	2	—	—	13	7	4
36	20	6	—	—	—	—	—
42	—	—	1.2	0.5	—	—	—
62	10	2	—	—	—	—	—

[a] Leachate contained suspended material.
[b] Based on ^{14}C.

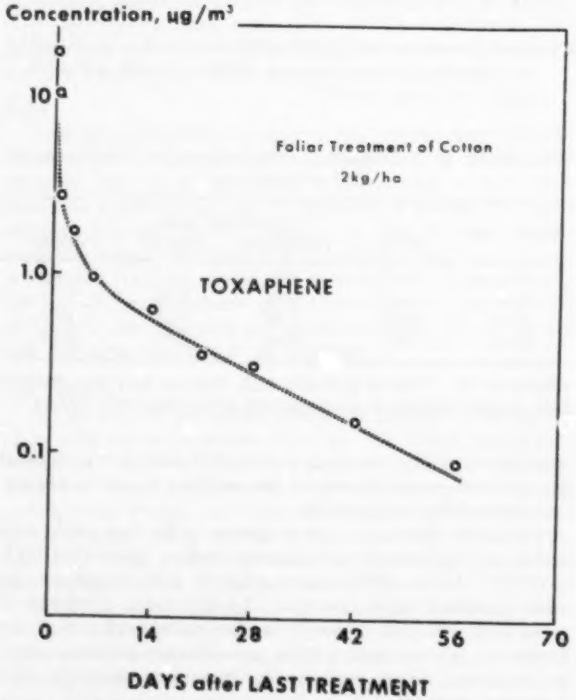

Figure 66. Concentration of toxaphene in air after a 2 kg/ha rate application of emulsifiable concentrate formulation to cotton in microagroecosystem.

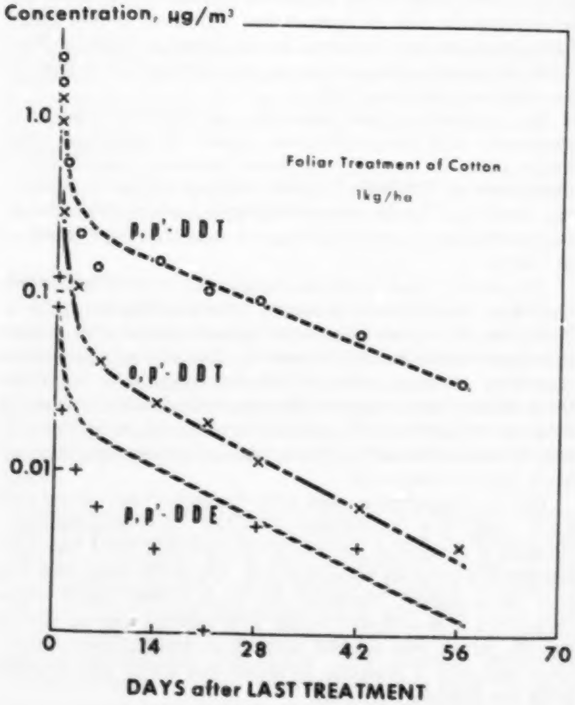

Figure 67. Concentration of DDTR residues in air after a 1 kg/ha rate application of emulsifiable concentrate formulation to cotton in microagroecosystem.

leached through the soil than did either the highly insoluble insecticides and fungicides.

Air concentrations of pesticide residues are given in Figures 66 through 70. Both insecticides (Figures 66 and 67) volatilized more than the emulsifiable silvex herbicide (Figure 69), and silvex volatilized more than did the fungicides (Figure 68). Volatilization of silvex and 2,4-D in granular formulation was the lowest of all (Figure 70). We used the isooctyl ester herbicides in our experiment, which

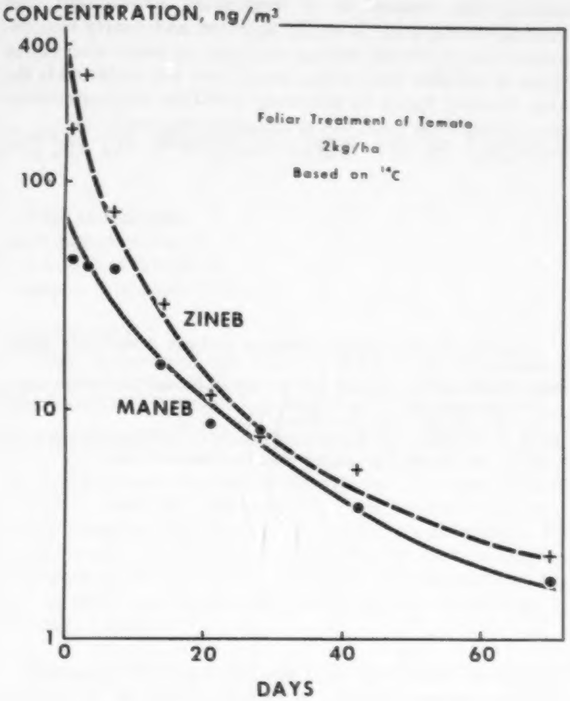

Figure 68. Concentration of maneb and zineb in air after a 2 kg/ha rate application of emulsifiable concentrate formulation to tomatoes in microagroecosystem.

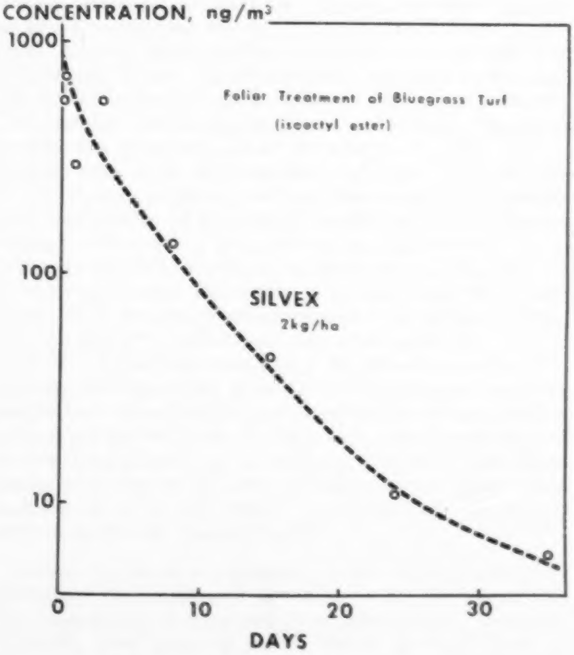

Figure 69. Concentration of silvex in air after a 2 kg/ha rate application of emulsifiable concentrate formulation to bluegrass turf in microagroecosystem.

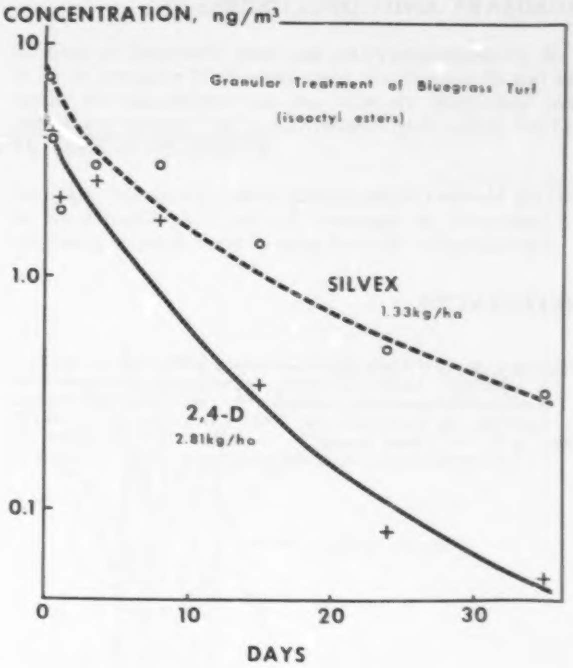

Figure 70. Concentration of silvex and 2,4-D in air after a 1.33 and 2.81 kg/ha rate, respectively, application of granular fertilizer formulation to bluegrass turf in microagroecosystem.

are low volatility esters. Our results demonstrated their low volatility as compared with the chlorinated hydrocarbon insecticides, and indicated that, from similar emulsifiable concentrate formulations, volatilization of p,p′-DDT from cotton (Figure 67) and isooctyl ester silvex from turf (Figure 69) were comparable. The method of analyses for these herbicides is for the acid only. Therefore, we could only assume the isooctyl ester was the major volatile product and not the acid.

Volatilization of the chlorinated hydrocarbon insecticides, like toxaphene and DDTR, has been demonstrated as a probable major pathway of dissipation only in the past 10 years. Volatilization of toxaphene was consistently more than double that for DDTR, even though the application was only double.

Volatilization from the granular formulations of 2,4-D and silvex and the fungicides was very low. The results for the fungicides zineb and maneb are only preliminary and based on their ^{14}C content. The methods of detecting the ethylenebis(dithiocarbamate) fungicides do not distinguish the parent compounds from the degraded products. However, because of the very low residue amounts trapped, we must assume that the fungicides did not degrade to the ethylene ^{14}C-labeled portion of the molecule, or the ^{14}C content would have been much higher.

Concentrations of toxaphene and DDTR in air decreased very rapidly for the first 7 days. We, presently, cannot satisfactorily explain this phenomenon. After about 7 days, the volatilization rate seemed to follow a log concentration with linear time relationship. The fungicides and herbicides volatilization rates did not quite follow this relationship. Neither do they follow a log concentration with log time relationship.

SUMMARY AND CONCLUSIONS

A microagroecosystem has been developed to monitor the fate of pesticides of a simulated field situation. A set of five individual systems can be constructed for about $11,000. Other than establishing a new experiment or discarding the wastes of a terminated experiment, the microagroecosystem is easily operated and nearly maintenance free. Pesticide residue analyses of many and varied types of samples (soil, plant, water, and air) collected is the only limiting factor in obtaining pesticide environmental-fate information from the microagroecosystems.

NOTE: Mention of proprietary products does not imply endorsement or approval by the U.S. Department of Agriculture to the exclusion of other suitable products.

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Fate of Pesticides in a Soil-Plant Microcosm

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ABSTRACT

A model microcosm, consisting of different soil layers, soil types, crop plants and percolating water is described. This system was used to study the fate, penetration and translocation into crop plants, movement with or without water and the metabolism of ^{14}C-insecticides under various soil and other environmental conditions. The interaction of different pesticide chemicals in this system was also studied, such as the effects of Eptam and its antidote on the uptake and metabolism of (^{14}C)Phorate in corn plants. Problems of unextractable or bound ^{14}C-residues in soils and in crops are discussed as well as the mechanism of binding in soils.

Studies pertaining to the behavior and fate of insecticide residues in the environment have usually been carried out with isolated components. Thus, many investigations have been conducted with a particular compound applied to soil, water, or to plants and with subcellular components of plants. Only recently have insecticides been studied in multi-component systems involving a variety of environmental factors (Metcalf et al. 1971). In some of these studies, behavior and deposition of pesticides were investigated by assembling isolated components of the environment into a more unified laboratory "ecosystem" or "microcosm." In our laboratory at the University of Wisconsin, the movement and metabolism of aldrin, parathion and (^{14}C) fonofos were studied in treated and untreated layers of several soil types relative to the effects of water moving through these soils. (Lichtenstein et al. 1967, 1972). Subsequently we employed a more complete ecosystem in which corn was planted in soil freshly treated with (^{14}C)-phorate through which water was percolated (Lichtenstein et al. 1974). As shown in Figure 71, studies were conducted with a model microcosm that consisted of soils, crop plants, and water. With this entity we attempted to investigate the interrelationships of movement and metabolism of (^{14}C) insecticides in different soil types, the effects of water percolating through these soils on these processes, and the penetration, translocation, and metabolism of insecticides in crop plants that grew in these soils under leaching and nonleaching conditions. In addition, this microcosm lends itself to study interaction phenomena of insecticide residues in soils with other "environmental" chemicals, such as herbicides, fungicides, etc.

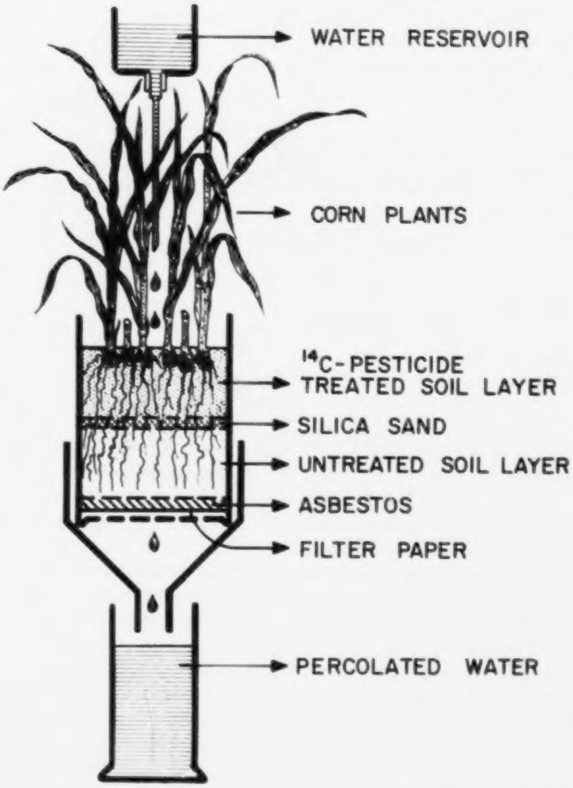

Figure 71. Microcosm used for the study of the movement, metabolism, and translocation of (^{14}C) pesticides in soils, plants and water.

Since, in these experiments, plants were grown immediately after treatment of the soil with the insecticide, the degradation of more persistent insecticides during the relatively brief growing period would be insufficient to assess the behavior of degradation products in this ecosystem. Therefore, another study was conducted with both freshly deposited insecticide soil residues and with residues aged for 6 months. The insecticide used as a model was Stauffer N-2596 (O-ethyl-S-(p-chlorophenyl) ethylphosphono-dithioate), which is very similar in structure to fonofos (O-ethyl-S-phenyl ethylphosphonodithioate). N-2596 has shown potential as an effective soil insecticide for the control of cutworms, wire worms, onion maggots, and other soil borne insect larvae (Harris and Svec 1970, Harris 1970, Day and Crosby 1972). Since previous studies in our laboratory had been conducted in a similar soil type with (^{14}C)-fonofos (Lichtenstein et al. 1972) which has a water solubility of 13 ppm, it was of interest to use a structurally similar compound (N-2596) but with a water solubility of only 3 ppm. We were also in a position to compare data obtained with N-2596 with that obtained with the organophosphorus insecticide (^{14}C)-phorate (Lichtenstein et al. 1974), which has a water solubility of 50 ppm. In comparison to both fonofos and phorate, and based on the

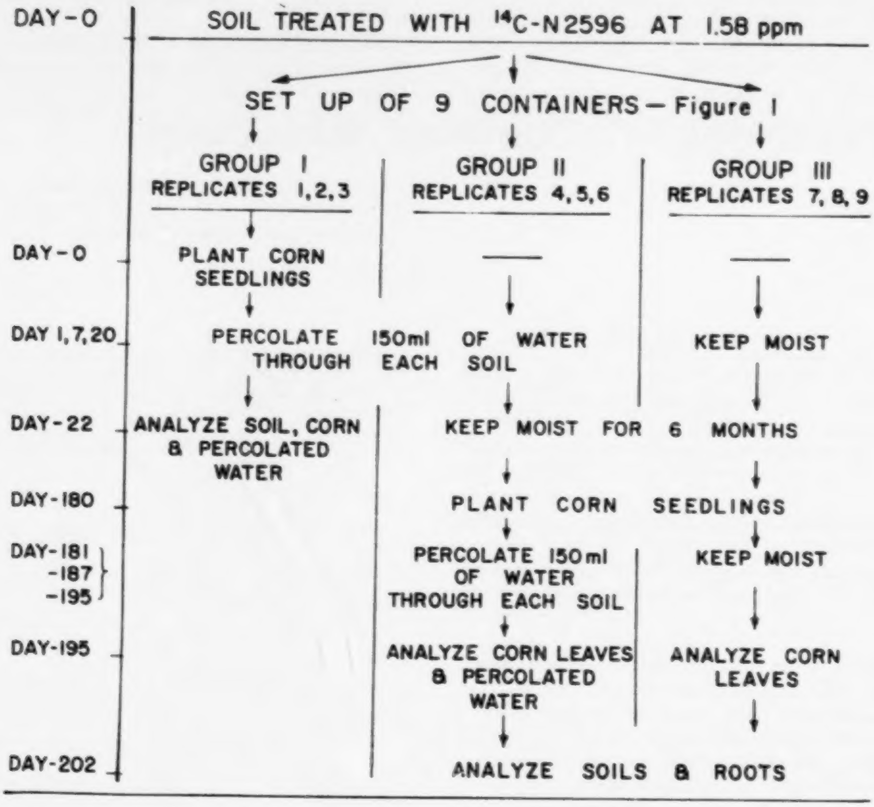
DAY-0
SOIL TREATED WITH ^{14}C-N2596 AT 1.58 ppm
SET UP OF 9 CONTAINERS — Figure I
GROUP I
REPLICATES 1, 2, 3
GROUP II
REPLICATES 4, 5, 6
GROUP III
REPLICATES 7, 8, 9
DAY-0
PLANT CORN SEEDLINGS
DAY 1, 7, 20
PERCOLATE 150ml OF WATER THROUGH EACH SOIL
KEEP MOIST
DAY-22
ANALYZE SOIL, CORN & PERCOLATED WATER
KEEP MOIST FOR 6 MONTHS
DAY-180
PLANT CORN SEEDLINGS
DAY-181
-187
-195
PERCOLATE 150ml OF WATER THROUGH EACH SOIL
KEEP MOIST
DAY-195
ANALYZE CORN LEAVES & PERCOLATED WATER
ANALYZE CORN LEAVES
DAY-202
ANALYZE SOILS & ROOTS

Figure 72. Flow sheet depicting the experimental design.

established water solubilities of these chemicals we expected N-2596 to be more persistent in soil, to show less mobility and be translocated and metabolized less readily. Probable differences in binding patterns or the appearance of unextractable insecticide residues also could be expected. Thus, we investigated metabolism and movement of (^{14}C)-N-2596 in our microcosm with freshly deposited and aged insecticide soil residues and also investigated the potential effects of percolating water on the fate of the insecticide in soils and corn plants.

Basically, 3 groups of experiments were conducted as shown in the outline of the experimental design (Figure 72). In this way, we were able to test the translocation and metabolism of (^{14}C)-N-2596 in a soil-corn-water ecosystem immediately following insecticide soil treatment (Group I, Figure 72), and with aged residues 6 months after the insecticide soil treatment (Group II, Figure 72). Group III (Figure 72) served as a control for testing the importance of percolating water since the soil was only kept moist during the 202 day incubation period. To measure the translocated amount of freshly deposited (^{14}C)-N-2596 and its metabolism, only soils in the 1st Group (Replicates 1, 2, and 3) were each planted with 8 germinated corn seeds. These and the 2nd group of soils (Replicates 4, 5, and 6) were then slowly percolated with distilled water 1, 7, and 20 days after the insecticide treatment to determine if the presence of growing plants would alter the amount of downward movement of the (^{14}C)-N-2596 caused by the percolating water. After 150 ml of water had been collected from each of the 6 containers during each percolation, aliquots were removed for bioassay with mosquito larvae to determine potential insecticide activity and for liquid scintillation counting to determine the amount of radioactivity present. The remaining water was then extracted with benzene and analyzed. After 22 days of growth, when the corn greens in Group I had reached a height of ca. 30 cm, shoots were cut 1 cm above the soil, then extracted and analyzed. The soils and roots in these containers (Replicates 1, 2, and 3) were allowed to dry for 6 days, when they were separated and removed as described (Lichtenstein et al. 1974).

To obtain aged insecticide residues the remaining 6 soils in which no corn plants had grown (Group II which had been percolated on days 1, 7, and 20 and Group III which had been kept moist) were allowed to incubate undisturbed for 6 months, except for maintenance of their moisture levels. One hundred eighty days after soil treatment, corn seedlings were planted in the insecticide-treated layers of Groups II and III and grown as described for 15 days when leaves reached a height of ca. 30 cm. During this growth period water was percolated through the soils of Group II on days 1, 7, and 15 after planting or 181, 187, and 195 days after soil treatment with the insecticide. Soils in Group III were kept moist during the corn growing period. Corn shoots were cut 1 cm above the soil surface on the 15th day after planting or 195 days after soil treatment, followed by extraction and analyses. Soils containing root material were kept for 6 more days to allow the percolated soils in Group II to dry to field moisture levels, while those in Group III were kept moist. Two hundred and two days after soil treatment the containers were dismantled and the roots were separated from the soils as described (Lichtenstein et al. 1974). The soil layers and the roots were then separately extracted and analyzed.

Data indicated (Lichtenstein et al. 1977) that under the described laboratory conditions (^{14}C)-N-2596 did not disappear as rapidly from the soil as did the organophosphorus insecticides phorate and fonofos, that it had a half life of 6 to 7 months and that its mobility in soils was comparatively insignificant. This latter property could be of advantage from an environmental point of view, since contamination of water or plants grown in these soils is rather limited. In comparison to freshly treated soils, studies with 6-month oil residues of (^{14}C)-N-2596 showed that no qualitative differences in the insecticide metabolism were evident. Only an increase in the amounts of water-soluble and bound soil residues, coupled with a decrease of benzene-soluble compounds was noticed after six months. This, however, had no effect on the penetration, translocation and metabolism of the insecticide or its metabolites in corn plants, except that roots and in particular corn leaves, contained more water-soluble ^{14}C-compounds. It appears that the translocation and metabolism of the insecticide in the plant are primarily governed by internal, physiological factors. Percolation of water through these soils resulted in a removal or reduction of noninsecticidal water-soluble ^{14}C-compounds in comparison to nonpercolated moist soil. Conversely, significantly less (at the 0.5% level) soil-bound residues were detected in moist soils than in the percolated ones.

Interaction of Pesticide Chemicals in the Microcosm: Effect of Eptam and its antidote on the Uptake and Metabolism of (^{14}C)-Phorate in Corn Plants

Modern agricultural practices have made extensive use of insecticides and plant growth regulators to increase food production. As a consequence, many soils contain residues of the various chemicals and/or their degradation products. The effects of these residues on subsequent pesticide applications as well as the effects of simultaneous applications of mixtures of agricultural chemicals on their metabolism in soil and their potential uptake by plants must be investigated. While the fate of individually applied agricultural chemicals has been extensively studied, relatively few reports on the interaction of herbicides and insecticides are available.

This study was initiated to investigate the effects of the herbicide Eptam (S-ethyl dipropylthiocarbamate) on the persistence and metabolism of phorate in soil and on its uptake by corn seedlings. Eptam is an effective preemergence herbicide commonly used for the control of grasses and broad-leafed weeds but its phytotoxicity toward certain varieties of corn has necessitated the introduction of antidotes to minimize that effect. The compound N,-N-diallyl-2,2-dichloroacetamide (Stauffer R-25788) is one of these antidotes.

The experimental set-up consisted of 12 cylindrical ice cream cartons each containing 925 g of Plainfield sand (0.6% organic matter; 94% sand; 3% silt; 2.4% clay). Three of these cartons contained soil treated with 2 ppm of (^{14}C) phorate only (9 uCi per 925 g of soil), three with 2 ppm of (^{14}C) phorate and 5 ppm of Eptam, three with 2 ppm of (^{14}C) phorate and 1 ppm of antidote, and three with 2 ppm of (^{14}C)phorate, 5 ppm of Eptam, and 1 ppm of the antidote.

Immediately after soil treatment, eight germinated corn seeds were planted in each of the 12 test soils. After watering, the weight of each container was determined and maintained throughout the experiment by daily additions of distilled water. The containers were held for 18 days at 21-24°C under a bank of Gro-Lux lamps (Sylvania Electric Products, Inc.) on a 12-h photoperiod.

Plant Harvest and Sample Preparation

Eighteen days after planting, corn greens had grown to a length of 25-29 cm. No visible plant injury could be observed. Leaves were cut 1 cm above the soil surface and the total weight of the greens from each replicate was determined. The containers were then dismantled. The roots were carefully removed from the soil and rinsed with tap water to remove any adhering soil particles. The total weight of the roots from each replicate was also determined.

No differences in the growth, weight, or general appearance of the corn seedlings, due to the various soil treatments, were observed. The average maximum length and combined weight of the greens from the eight plants in each of the three replicates treated with phorate only were 28.9 ± 2.3 cm and 5.8 ± 1.1 g, respectively; with phorate and Eptam, 26.0 ± 0.6 cm and 5.6 ± 0.5 g, respectively; with phorate and the antidote, 26.5 ± 1.9 cm and 6.0 ± 0.6 g, respectively; and with phorate, Eptam, and the antidote, 24.8 ± 2.4 cm and 5.0 ± 0.9 g, respectively. The weights of the roots from 8 plants per container varied between 8.0 and 10.5 g. After extraction and analyses of soils and plant material, the interaction of Eptam with plants in affecting the penetration, translocation and metabolism of ^{14}C-phorate in corn was indicated (Schulz et al. 1976). While at concentrations of 5 ppm of Eptam in a (^{14}C)phorate treated (2 ppm) Plainfield said no visible damage to the growing corn plants was noticeable, the amounts of ^{14}C compounds in corn greens from herbicide-treated soil were significantly increased by a factor of 1.8. In particular, the amount of bound, unextractable ^{14}C-labeled residues was higher in plants grown in (^{14}C)phorate plus Eptam-treated soil. After 18 days soils contained phorate, phorate sulfoxide, and phorate sulfone while in greens only the metabolites phorate sulfoxide, phorate sulfone, and phoratoxon sulfoxide could be detected. Due to the presence of Eptam in the (^{14}C)phorate-treated soil, the amounts of both phorate sulfoxide and phorate sulfone were significantly increased by a factor of 1.6 and those of phoratoxon sulfoxide by a factor of 2.4 (Figure 73). Addition of the antidote (at 1 ppm) to (^{14}C)phorate-treated soil had no effect on the translocation and metabolism of insecticides in corn greens, yet counteracted the effects of Eptam. Thus, analyses of corn greens grown in (^{14}C)phorate-treated soil containing also Eptam and its antidote gave results similar to those grown in soil which had only been treated with (^{14}C) phorate. These findings further point to the problem of potential interactions of environmental chemicals in biological systems.

Binding Phenomena of ^{14}C-insecticides in Soils

"Bound" or unextractable residues increased in soils with time. However, they were found to be identical in green portions of corn plants grown under the 3 experimental conditions described above. Further studies were conducted in our laboratory, to investigate binding phenomena and mechanisms of binding of ^{14}C-insecticides in soil.

Since the introduction of synthetic chemicals for agricultural pest control, difficult problems have emerged regarding their ultimate fate. Because of the limited analytical methodology before 1962, and the limited knowledge

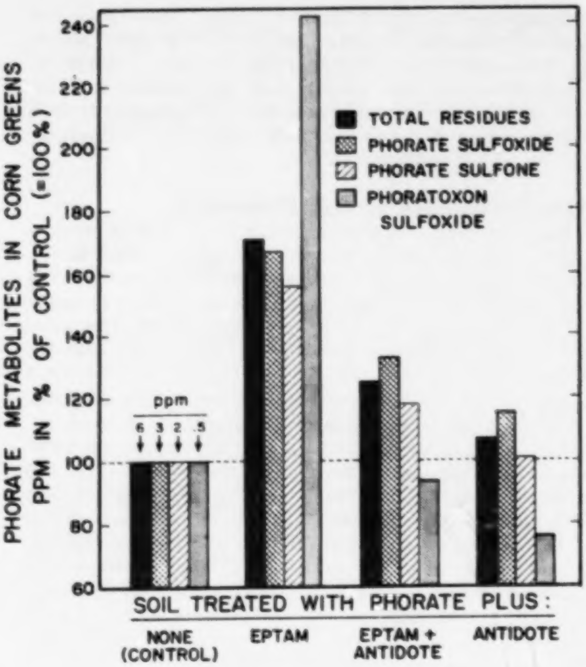

Figure 73. The effects of Eptam and its antidote on the translocation of phorate from a Plainfield sand. The soil had been treated with the insecticide at 2 ppm (control), with the insecticide and Eptam (at 5 ppm), with both plus the antidote (at 1 ppm), and with the antidote alone. Actual concentrations of insecticide metabolites in greens grown in soils containing only phorate (control) are indicated (parts per million).

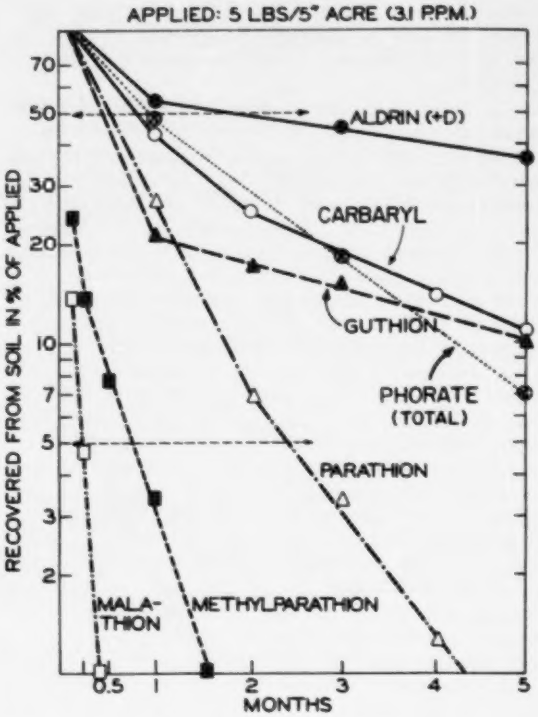

Figure 74. Depletion curves depicting extractable residues of various insecticides applied at 6 kg/hectare (concentration, 3.1 ppm) to loam soils under field conditions.

about the metabolism of many pesticides, analyses for these compounds were primarily conducted for the applied "parent" compound. In addition, only easily extracted residues were detected. Accordingly, typical depletion curves for several chlorinated hydrocarbon insecticides in soils were published by this laboratory, showing the "persistence," "disappearance" or "loss" of the pesticides (Figure 74), such as the relatively short persistence of parathion residues in soils under both laboratory and field conditions. Whenever the fate of pesticide residues in the environment was described, the vague terms "disappearance" or "volatilization" were widely used, which did not consider the formation of unextractable, bound residues. It was only after the introduction of radiolabeled pesticides for experimental use that it was possible to obtain a "balance" and to account for the fate of the applied radiocarbon. The use of combustion or strong hydrolytic techniques with the extracted soils revealed that unextracted or bound residues could be released and detected. The problem of the bound residues, however, is complicated since present methods for their release or liberation (combustion, hydrolysis) also result in the destruction of their identity. Insight into the mechanism of binding of pesticide residues to soils might shed some light on the nature of the residues and their potential release.

Utilizing ^{14}C-ring and ^{14}C-ethoxy labeled parathion, the amount of unextractable or bound ^{14}C-residues in a sandy and a loam soil were determined by combustion to $^{14}CO_2$ after the soils had been extracted. Depletion curves of extractable parathion residues established during a 1-month incubation period were similar to those established under field conditions and resulted finally in recoveries of 30 to 36% of the insecticide dose applied to the loam soil. With a steady decrease of extractable residues over a one-month incubation period, an increase of unextractable, bound residues occurred. This resulted finally in total recoveries of extracted plus bound residues, which amounted to 80% of the applied radiocarbon (Figure 75). Soils containing bound residues were non-toxic to fruit flies. The rate of binding of ^{14}C-residues was highest in the loam soil and was related to the activity of soil microorganisms. In soils, sterilized by gamma irradiation or by autoclaving, the binding of ^{14}C-parathion was reduced by 58 to 84%. Under anaerobic conditions, created by flooding soils with water, the rate of binding of 14C-compounds doubled, but the amount of bound residues decreased from 67% to 16% when soils had been sterilized prior to the insecticide treatment and flooding. Reinoculation of this soil with microorganisms fully reinstated the soil binding capacity. The pronounced binding under anaerobic conditions was associated with a reduction of parathion to aminoparathion. The reduced compound was preferentially bound to soil; after soil treatment with ^{14}C-aminoparathion or with ^{14}C-parathion, the amount of ^{14}C-aminoparathion bound to the soil in 2 hours was 30 times greater than the amount of ^{14}C-parathion bound to the soil during the same incubation period (Katan et al. 1976).

The role of microorganisms in producing ^{14}C-Parathion derived bound residues and the mechanism of production of

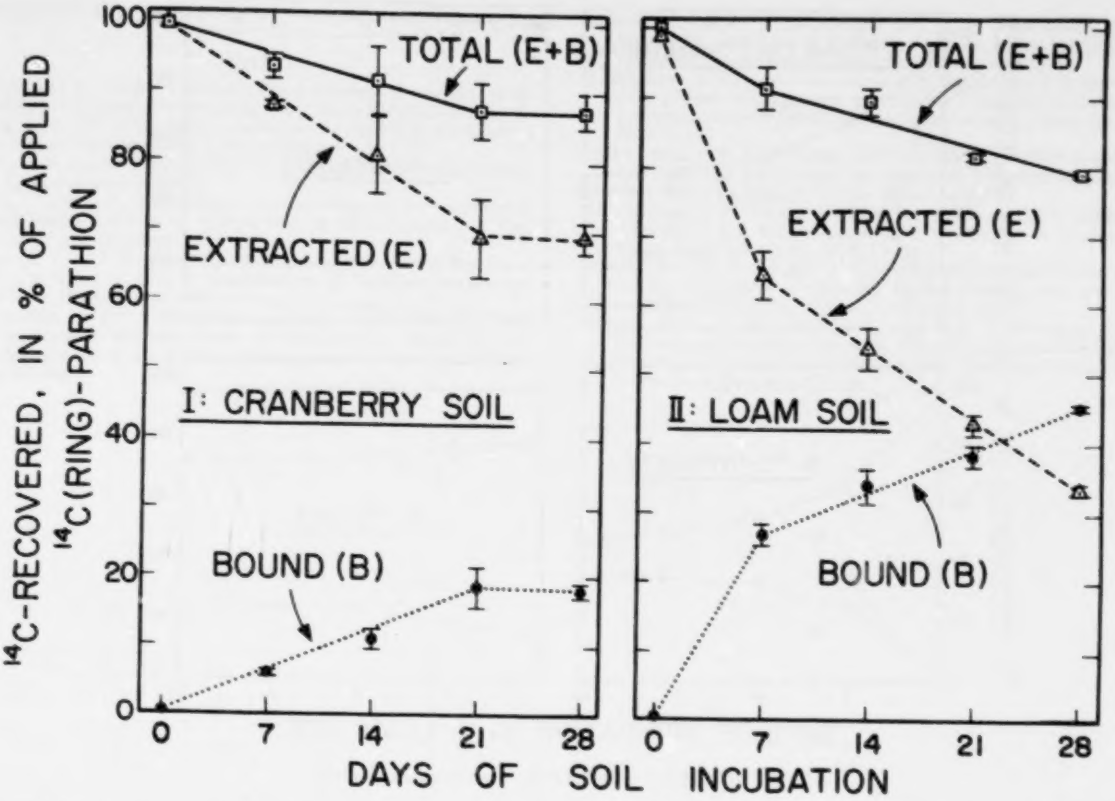

Figure 75. Binding and extractability of (phenyl-^{14}C)parathion in two soils during a 28-day incubation period at 27°C (applied dose, 1 ppm). The amounts of parathion as determined by GLC in the loam soil extract were nearly identical to its radiocarbon content. (I) Soil from cranberry bog; (II) loam soil. Curve B, bound parathion; curve E, extracted parathion; and curve E + B, the total.

soil bound residues was also investigated by incubating ^{14}C-(ring)-parathion in soil-free culture media that had been inoculated with soil microorganisms. The amount of ^{14}C-compounds in culture supernatants, that upon addition to soil became unextractable, increased up to 12 hours of microbial culture incubation, when 43% of the added radiocarbon was bound after a 2-hour soil incubation period. The increase in soil bound residues was correlated with a decrease in the amount of parathion in the microbial culture and a concomitant increase in the appearance of the major degradation product, aminoparathion (Figure 76). Microbial cells contained only a minor proportion of ^{14}C-bound residues. Addition of natural microbial growth metabolites to the soil did not affect binding of either parathion or aminoparathion. When ^{14}C-parathion, ^{14}C-paraoxon and ^{14}C-p-nitrophenol and their respective ^{14}C-amino compounds were added to soil the amino-compounds were bound to a much greater extent that were the nitro-compounds. It appears that the process of parathion degradation has to be separated from that of binding and that the role of the microorganisms in soil binding phenomena consisted in degrading ^{14}C-parathion to compounds which are soil bindable. Once these compounds were formed, their binding to soil was found to be high (Katan and Lichtenstein, 1977). In light of our knowledge of the existence of formerly "unseen" unextractable residues, it appears that the concept of "persistent" and "non-persistent" pesticide residues should be reconsidered.

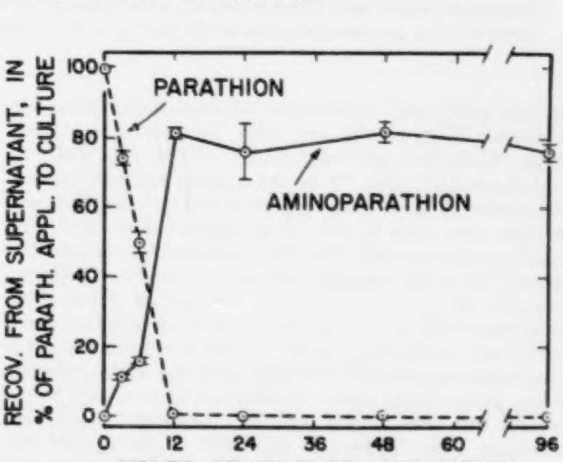

Figure 76. Amounts of parathion and amino-parathion recovered by gas liquid chromatography from benzene extracts of supernatants of microbial cultures after treatment with ^{14}C-ring-parathion at 10 ppm and incubation for 0 to 96 hours.

Further investigations (Lichtenstein et al. 1977) pertaining to the extractability and formation of bound ^{14}C-residues in an agricultural loam soil were conducted with

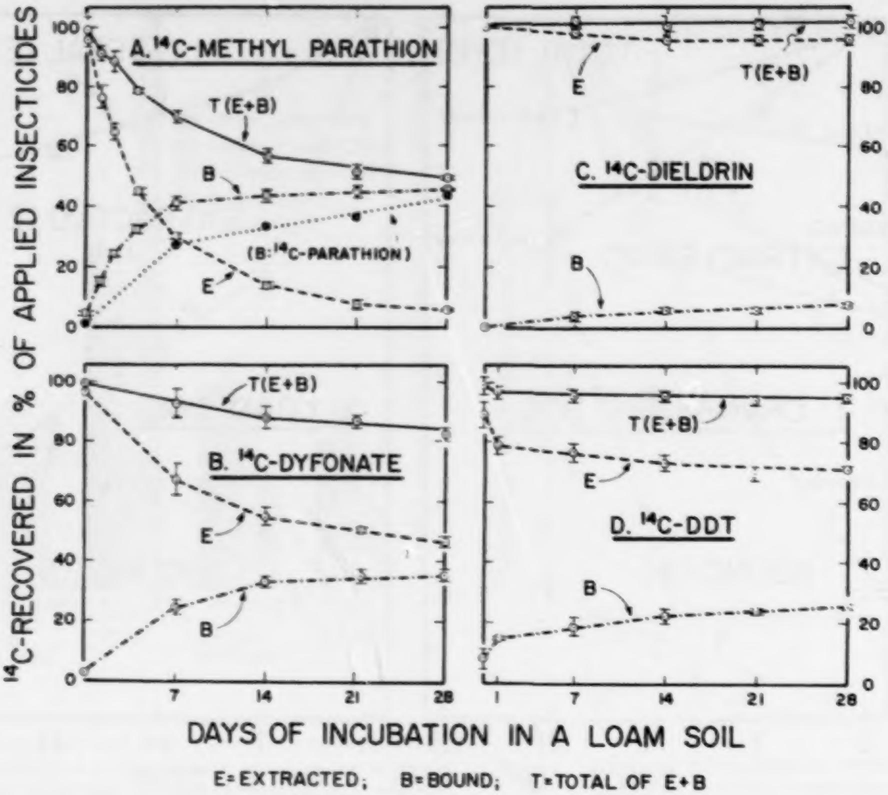

Figure 77. Binding and extractability of ^{14}C-labeled insecticides in a silt loam soil during a 28-day incubation period, after soil treatment at 1 ppm. With the exception of (^{14}C)methylparathion the amounts of extractable (^{14}C)Dyfonate (fonofos), (^{14}C)dieldrin, and (^{14}C)DDT, as determined by gas-liquid chromatography, were similar to the amounts of extractable radiocarbon. For comparison purposes, data are inserted in A for the bound residues of (^{14}C)parathion in soil (Katan et. al. 1976).

the "non-persistent" insecticides ^{14}C-methyl-parathion and ^{14}C-fonofos (Dyfonate®) and with the "persistent" insecticides ^{14}C-dieldrin and ^{14}C-p,p'-DDT (Figure 77). With ^{14}C-methylparathion only 7% of the applied radiocarbon was extractable 28 days after soil treatment, while ^{14}C-bound residues amounted to 43% of the applied dose. With ^{14}C-fonofos, however, still 47% of the applied dose was extractable and 35% of the applied radiocarbon was bound. Only a fraction of the radiocarbon extracted from ^{14}C-methylparathion treated soil was associated with the parent compound, while extractable ^{14}C-residues from the other insecticide-treated soils were primarily due to the presence of the parent compounds. Smaller amounts of soil-bound residues had been formed with the "persistent" insecticides amounting after 28 days to only 6.5% of the applied ^{14}C-dieldrin and to 25% of the applied ^{14}C-p,p'-DDT, while 95% and 72%, respectively were still recovered by organic solvent extraction. They differed from the organophosphorus compounds in their relatively low binding properties and their high extractability from soils. Contrary to results with ^{14}C-parathion, the mechanisms of binding of ^{14}C-fonofos was not dependent on the presence of soil microorganisms. At higher application rates of the insecticides, relatively less radiocarbon was bound, possibly due to saturation of binding sites.

The question of the potential biological activity of bound insecticide residues was investigated by testing the insecticidal activity of bound residues from (^{14}C) fonofos and (^{14}C) methylparathion treated soils with fruit flies. With soils containing unextractable radiocarbon at the insecticide equivalent of 3 ppm, no mortalities were observed during a 24-hour exposure period to the soil and only slight mortalities during an additional 48-hour exposure period. However, with soils to which the insects were exposed immediately following the insecticide application at the same concentration as the unextractable radiocarbon (3 ppm), 50% of the flies had died within 2-3 hours after fonofos application and within 18-20 hours after soil treatment with methylparathion. It appears, therefore, that bound residues are not only unextractable, but they are also less active biologically.

In the future, it will be important to obtain information about the mechanism of binding of pesticides, thus possibly shedding some light on the mechanism of their potential release and the conditions at which this release might occur. Since not much information is available pertaining to the nature and the potential biological activity of the compounds that are bound, extensive research in this field is highly desirable. In view of the above findings, the expression "disappearance" and "persistence" of pesticides, so widely used during the last two decades, should be reassessed to consider the bound products.

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Natural Systems

Effects of Substrate Type and Arsenic Dosage Level on Arsenic Behavior in Grassland Microcosms. Part I: Preliminary Results on ^{74}As Transport[a,b]

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ABSTRACT

Microcosm design is an important factor in interpreting results obtained from studies of the environmental effects, mobility and persistence of contaminants. The behavior of pentavalent arsenic and a radioarsenic tracer was studied in three substrate types exposed to differing levels of stable As dosage. Soil cores excised intact from a natural grassland ecosystem were considered to most reliably represent the natural system under study since they retained the soil structure, and closely simulated the abiotic and biotic complexity, of the grassland ecosystem. The behavior of ^{74}*As in the components of intact soil core microcosms differed appreciably from that observed for the other microcosm types where soil underwent manipulation. These differences were explained primarily on the basis of differences in soil structure.*

INTRODUCTION

Microcosms are small laboratory experimental units containing the major components, and exhibiting major processes, of a whole ecosystem. Conceptually, microcosms are functionally similar to, but may differ in origin and structure from the natural ecosystems that they mimic (Draggan, 1976a). They may be constructed artificially from stock components, or intact, co-adapted communities and their abiotic substrates may be excised from a natural system (Draggan and Giddings, 1977). Microcosm studies have been characterized specifically by their attention to the measurement of ecosystem processes in isolation (i.e., nutrient cycling and decomposition, energy flux and utilization, and population and community dynamics).

Passage of the Toxic Substances Control Act[d], which requires preproduction testing of chemicals, has increased interest in the use of microcosms to identify potential hazards to ecosystems receiving chemical inputs. To provide meaningful predictions of chemical hazard, microcosm studies must be designed to provide integrated information on chemical effects, mobility and persistence in ecosystems. To achieve this goal microcosms should closely simulate the structure of, and the processes occurring in, natural and perturbed ecosystems.

Research currently underway in the Environmental Sciences Division at Oak Ridge National Laboratory[e] has identified some problems encountered in interpreting results from previous microcosm studies of chemical behavior in aquatic and terrestrial environments. The major problem relates to the application of results from processes measured in isolated experimental units to processes actually occurring in more complex, interactive natural ecosystems. Also, the influence of microcosm size and structure (i.e., biotic and abiotic complexity) on the results obtained has not been adequately evaluated. Finally, there is limited information on the confidence limits (replicability) of measured parameters within experiments, and on the reproducibility and comparison of microcosm studies. Two factors recognized as important determinants of chemical behavior in microcosms, and that require further study to optimize the environmental realism of microcosm design and experimental procedures are substrate type (degree of natural substrate modification) and contaminant dosage level. This report provides a detailed methodology for the construction and use of terrestrial microcosms for evaluating the environmental behavior of chemicals, as well as information on the transport of radioarsenic in microcosms of a natural grassland ecosystem.

Substrate Type

Substrates utilized in many microcosm studies of chemical behavior in terrestrial environments have been artificial, or highly modified. Soil simulants have included sand (Draggan, 1976b; Witkamp and Frank, 1970; Metcalf, 1973), sand-soil mixtures (Gillett and Gile, 1976), potting soil (Gillett and Cile, 1976; Cole et al., 1976), and homogenized soil (Lighthart and Bond, 1976; Witkamp, 1976). The usual justification for use of artificial and homogenized soils has been enhancement of experimental replicability through reduction of system complexity and heterogeneity. However, use of artificial or highly modified soil simulants discounts the importance of natural soil structure (i.e., crumb size, porosity, profile development) and soil organism-soil interrelationships (i.e., microbes-soil, roots-soil, invertebrates-soil) to contaminant behavior. In addition, recent studies have demonstrated that excised, intact soil cores provide accurate, replicable, and inexpensive experimental units for contaminant assessment (O'Neill et al., 1977).

Dosage Level

Typically, in studies of chemical effect, contaminants are introduced into test systems at a dosage, or range of dosages, expected to elicit a response. However, in microcosm studies of potential chemical hazard, the optimal concentration (ideally a concentration approximating that actually entering the environment) and any concentration dependent effects on mobility or persistence must be determined (Draggan and Giddings, 1977).

To evaluate the utility of microcosms in chemical testing schemes an experiment was designed to determine the effects of substrate type and contaminant dosage level on the transport and localization of a contaminant in microcosms representing a natural grassland ecosystem. For this experiment, intact, excised soil cores were expected to most accurately represent the natural ecosystem because many features of natural system complexity and structure are retained.

MATERIALS AND METHODS

Ecosystem Studied

It is expected that the ultimate sink of many chemicals released to the biosphere will be soils of terrestrial ecosystems (i.e., landfills, agricultural lands, grasslands, and forests) (Loehr, 1977). The ecosystem type chosen for study was a managed grassland within the ERDA Reservation in Oak Ridge, Tennessee. The predominant vegetation of the area is *Festuca elatior* (Van Hook et al., 1970; Matti et al., 1975). The soil is a Captina silt loam (bulk density 1.21 g/cm^3). It is well drained, fairly fertile (3.7% organic matter) and slightly acidic (pH 5.4-5.6). The soil has been physicochemically characterized for some nutrient elements (Kelley et al., 1969). Mean annual precipitation for the area is ~124 cm and is evenly distributed throughout the year (Holland, 1953).

Contaminant Studied

Pentavalent arsenic was the contaminant chosen for study. Major sources of arsenic contamination are pesticides and metal smelting operations. Minor sources of arsenic include: (1) coal combustion, (2) kaolin clay products used in paper manufacture, (3) super-phosphate fertilizers, (4) acid mine drainage, (5) wood preservatives, and (6) wastes from wool processing (Biomedical Studies Group, 1976). Pentavalent forms of arsenic are less toxic than the trivalent forms. Under reducing conditions (i.e., anaerobic soils and sediments) microorganisms may methylate arsenic compounds forming volatile, highly toxic species of arsenic (i.e., mono-, di-, and tri-methylarsines). Arsenic is readily converted from one oxidation state to another by chemical and biotic processes making the environmental chemistry of arsenic and its compounds extremely complex (Wood, 1974; Biomedical Studies Group, 1976).

Microcosm Substrate Types

Three substrate types, commonly used in microcosm studies of chemical behavior, were selected to compare substrate effects on the localization and transport of radioarsenic added to models of the grassland ecosystem. The substrate types were:

(1) excised, intact soil cores (CSL) containing aboveground vegetation, roots, and co-adapted populations of soil organisms, and retaining natural soil structure.

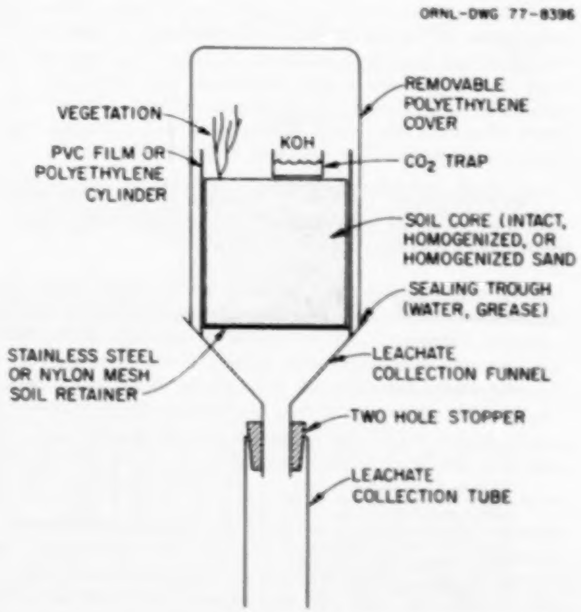
(2) homogenized soil (HOM), containing soil microbes and small soil invertebrates with soil structure disrupted. The crumb size of this substrate was ~1.0 - 2.0 mm which allowed formation of numerous soil water channels.

(3) 10% homogenized soil: 90% Ottawa sand cores (W:W). Thorough mixing of the components created a very finely divided (high surface area) soil. Designated SSC soil.

Microcosm Design

Intact soil cores (CSL) and soil for the fabrication of HOM and SSC microcosms were collected from a relatively level, floristically uniform area of the grassland ecosystem. The intact cores (5 cm diameter x 10 cm depth) and soil collected to a depth of 5 cm were bagged in polyethylene to minimize dessication and returned to the laboratory. The intact cores were cut to a 5 cm depth and sheathed with cylinders of shrinkable polyvinylchloride (PVC). The PVC sheathed cores were then gently heated until a tight bond with the vertical surfaces and bottom of the cores was established.

Soil collected as the grassland ecosystem was sieved (2.0 mm mesh opening) to provide uniform crumb size. Homogenized soil was packed to a depth of 5 cm in stainless steel mesh bottomed polyethylene cylinders (5 cm diameter) to form the homogenized (HOM) set of microcosms. The remaining homogenized soil was then mixed with acid washed-distilled water rinsed Ottawa sand and packed into identical polyethylene cylinders to a depth of 5 cm. Packing of the cylinders entailed a settling process, the soil was not tamped, allowing the bulk density of the HOM and SSC substrates to remain constant with depth. All cores were masked with opaque electrical tape and attached to leachate collection—support systems (Figure 78).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VEGETATION
PVC FILM OR POLYETHYLENE CYLINDER
KOH
REMOVABLE POLYETHYLENE COVER
CO_2 TRAP
SOIL CORE (INTACT, HOMOGENIZED, OR HOMOGENIZED SAND
SEALING TROUGH (WATER, GREASE)
STAINLESS STEEL OR NYLON MESH SOIL RETAINER
LEACHATE COLLECTION FUNNEL
TWO HOLE STOPPER
LEACHATE COLLECTION TUBE

Figure 78. Leachate collection and support system for laboratory microcosms used to determine arsenic transport.

Maintenance During Equilibration

All microcosms were allowed to equilibrate for two months before the contaminant and tracer were added. During this period microcosms were maintained in an environmental chamber with diurnal light:temperature regime (12 hrs dark: 20 C; 12 hrs light: 27 C) and relative humidity of ~ 95%. The microcosms were watered weekly using 20-ml aliquots of aerated rain water throughout the experiment. Leachates produced during this period were reintroduced into respective microcosms to minimize nutrient depletion. of CO_2 efflux for 4 hours each week using 0.18 N KOH as the CO_2 trapping agent (Conway, 1962). The amount of CO_2 evolved was measured titrimetrically and reported as mg CO_2/hr/microcosm.

Contaminant Amendment

Stable pentavalent arsenic (as Na_2HAsO_4) at dosages of 0 ppm, 10 ppm, and 100 ppm As was added to treatment microcosms following equilibration. At each stable arsenic dosage level pentavalent $^{74}AsO(OH)_3$ was added concurrently with the stable amendment at ~ 3.22 x 10^7 dpm (~ 2.33 ppb) as a tracer for stable As movement. Each treatment microcosm was dosed with the arsenic contaminant and tracer in 10 ml of distilled water. The ionic forms of the stable and radioactive amendments were identical since in aqueous solution sodium arsenate hydrolyzes to $AsO(OH)_3$.

Experimental Design and Analysis

The major components of all microcosms used in this study were leachates, soils, and soils exported from microcosms during leaching. Leachates were filtered (Whatman #40) to remove exported soils, and radioarsenic activity lost from microcosms in leachates and in exported soils was measured for 30 days at approximately weekly intervals. Radioanalysis of these components provided information on ^{74}As loss over time and total ^{74}As loss. Results from the radioanalysis of exported soils were pooled with the results for total ^{74}As activity in the soils of respective microcosms. Microcosm substrates were radioassayed at the conclusion of the experiment to determine soil profile distribution and total soil retention of ^{74}As. The behavior of ^{74}As was expressed as the percent of the initial activity recovered in microcosm components. All radioisotope data were corrected for decay, background and counting efficiency and analysed by two-way analysis of variance.

The experiment followed a factorial design with main effects of substrate type and stable As dosage level according to the following scheme:

STABLE As DOSAGE	SUBSTRATE TYPE		
	CSL	HOM	SSC
NO STABLE As:NO As (0-0)	——— ——— ——— ——— ———	——— ——— ——— ——— ———	——— ——— ——— ——— ———
NO STABLE As: ^{74}As (0-*)		etc.	
10 ppm STABLE As: ^{74}As (10-*)		etc.	
100 ppm STABLE As: ^{74}As (100-*)		etc.	

15 control microcosms
45 treatment microcosms
60 microcosms total

RESULTS

The experimental design allowed determination of both stable and radioactive arsenic distribution in microcosm components over time and at the conclusion of the experiment. To date only the distribution of ^{74}As in the microcosm components has been determined.

Radioarsenic Loss from Microcosms in Leachates

Most of the radioarsenic loss from microcosms in leachates occurred on the first watering-leachate collection date (Figures 79, 80, 81). Radioarsenic loss in leachates on subsequent collection dates was nil. Total loss of ^{74}As was significantly different ($P \leq 0.0001$) for all substrate types in the order HOM > CSL > $\overline{S}$SC (Figure 82a). A dosage effect on ^{74}As loss was observed wherein microcosms receiving no stable As lost lower amounts of ^{74}As than those receiving 10 ppm stable As and significantly ($P < 0.02$) lower ^{74}As loss than those dosed with 100 ppm As (Figure 82b). Substrate type and stable As dosage level interacted significantly ($P \leq 0.001$) indicating non-independence between these variables. Dosage effects were inconsistent between microcosm types since the substrates cannot be compared quantitatively (Figure 82). HOM microcosms demonstrated, over a wide range, an effect of increasing ^{74}As loss with increasing stable As dosage level. A similar trend was observed for SSC microcosms; and, in this case total ^{74}As loss was linearly related to As dosage level (between 0 and 100 ppm As) according to the function:

^{74}As LOSS (%) = 0.02 (DOSAGE LEVEL) + 0.06; (r^2 = 0.99).

CSL microcosms demonstrated the opposite trend of decreasing ^{74}As loss with increasing stable As dosage level.

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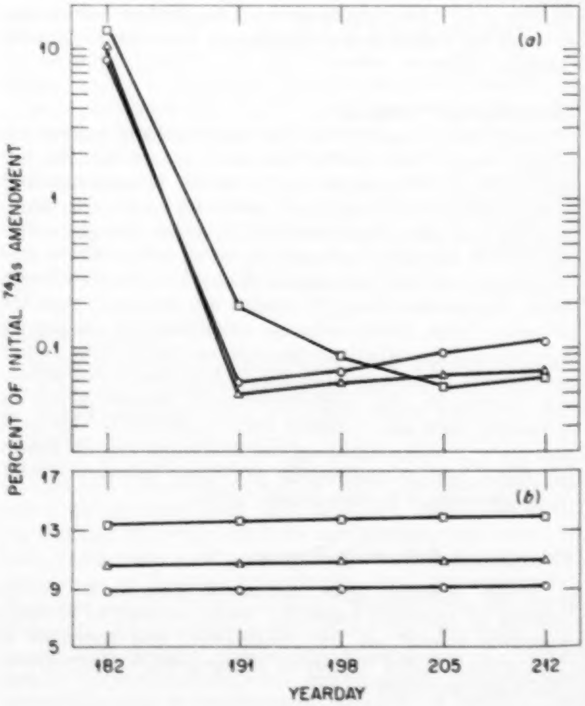

Figure 79. (a) Percent of the initial ^{74}As amendment recovered in leachates from CSL microcosms. □ – < 1 ppm As, Δ – 10 ppm As, and ○ – 100 ppm As. (b) Cumulative plot of the data.

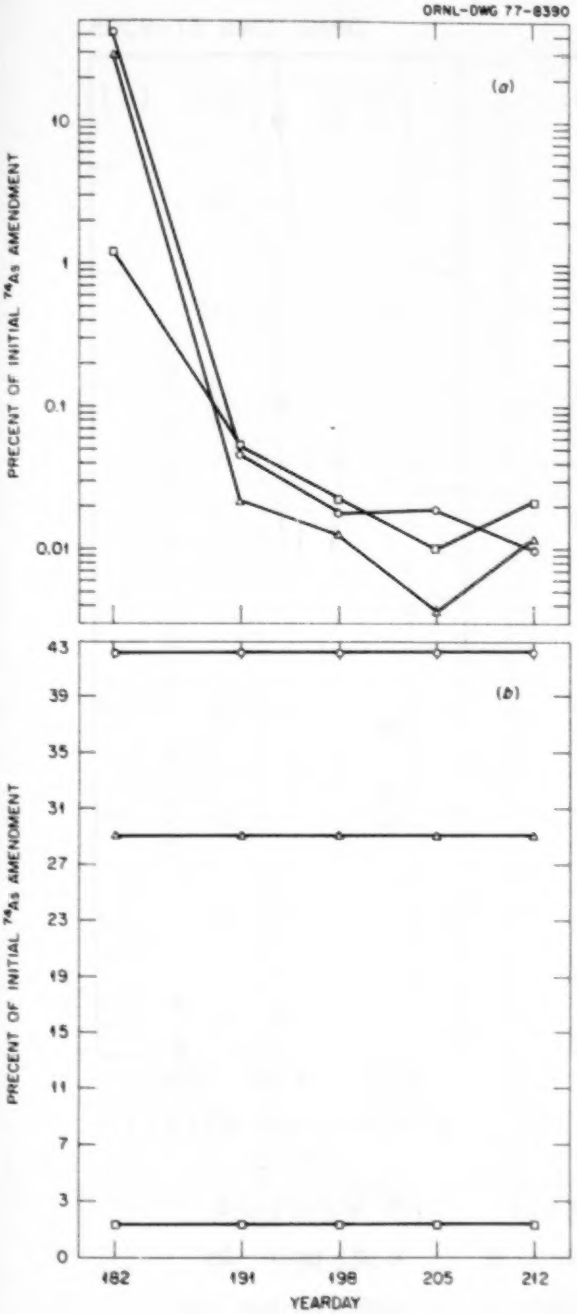

Figure 80. (a) Percent of the initial ^{74}As amendment recovered in leachates from HOM microcosms. □ – < 1 ppm As, Δ – 10 ppm As, and ○ – 100 ppm As. (b) Cumulative plot of the data.

Radioarsenic Retention in Microcosm Substrates

The amount of ^{74}As retained in the total soil component of microcosms was significantly different ($P \leq 0.0001$) for all substrate types with retention following the order SSC > CSL > HOM (Figure 83a). SSC microcosms retained ~ 20% more ^{74}As than CSL microcosms, while CSL microcosms retained ~ 24% more ^{74}As than HOM microcosms in the total soil component. There was no significant effect of As dosage level on ^{74}As retention in microcosm substrates; however, overall soil retention of ^{74}As decreased with increasing dosage level (Figure 83b). Substrate type and As dosage level again demonstrated significant interaction ($P \leq 0.004$). CSL microcosms exhibited increasing ^{74}As retention with increasing As dosage level. HOM microcosms, and to a lesser extent SSC microcosms, demonstrated the opposite trend (Figure 83c).

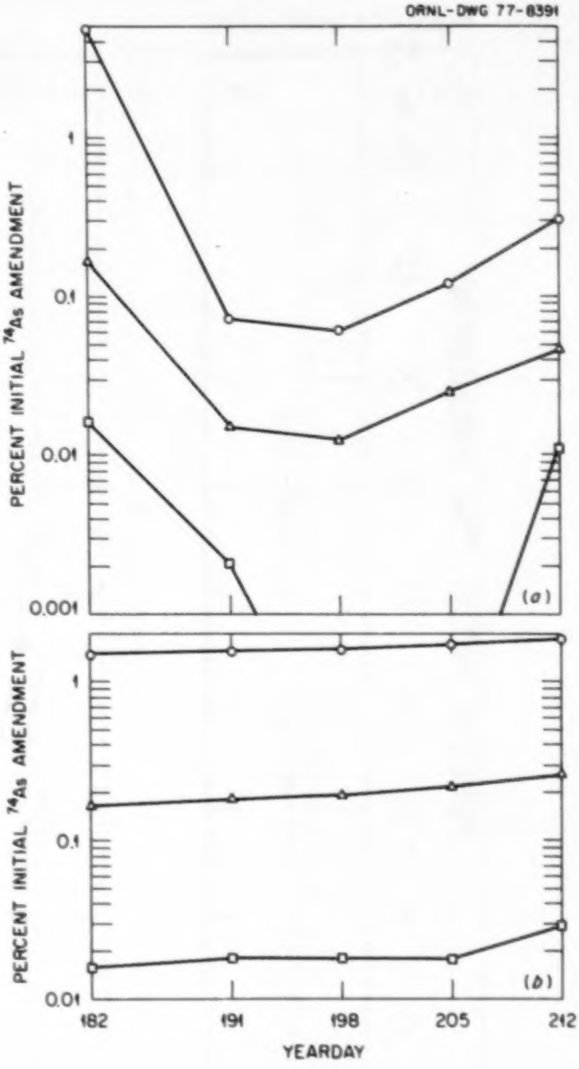

Figure 81. (A) Percent of the initial ^{74}As amendment recovered in leachates from SSC microcosms. □ – < 1 ppm As, Δ – 10 ppm As, and ○ – 100 ppm As. (b) Cumulative plot of the data.

In addition to determination of ^{74}As retention in the total soil component of microcosms, ^{74}As retention as a function of depth was determined by radioanalysis of one cm increments of substrate sample cores. Between 50%-98% of the ^{74}As was retained in the uppermost one cm of substrate. The different substrate types exhibited significant differences ($P \leq 0.004$) in ^{74}As retention in the uppermost

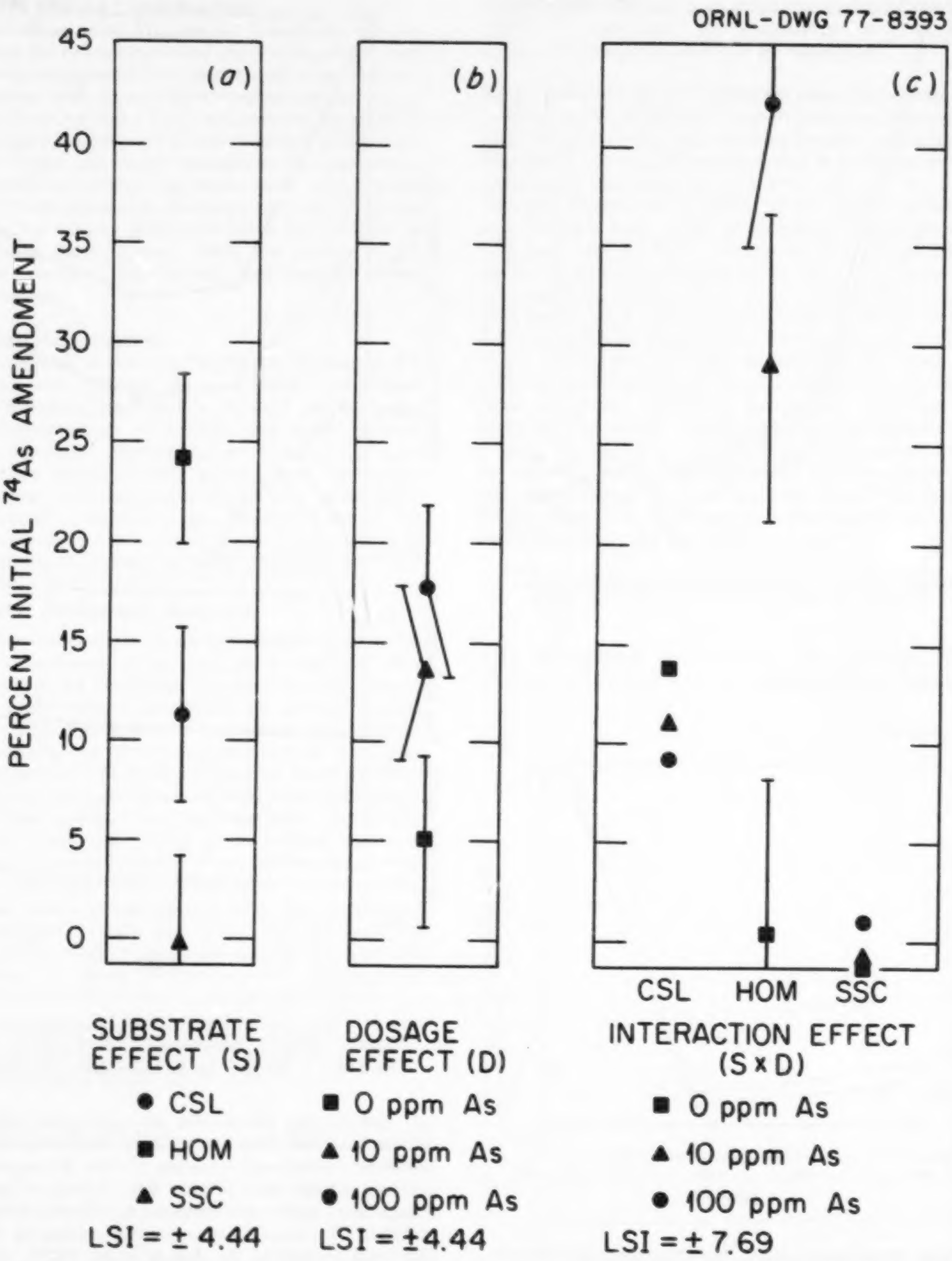

Figure 82. The effects of substrate, As dosage level and their interaction on the percent of the initial ^{74}As amendment recovered in leachates from microcosms. Least significant intervals are presented when means are significantly different ($P \leq 0.05$).

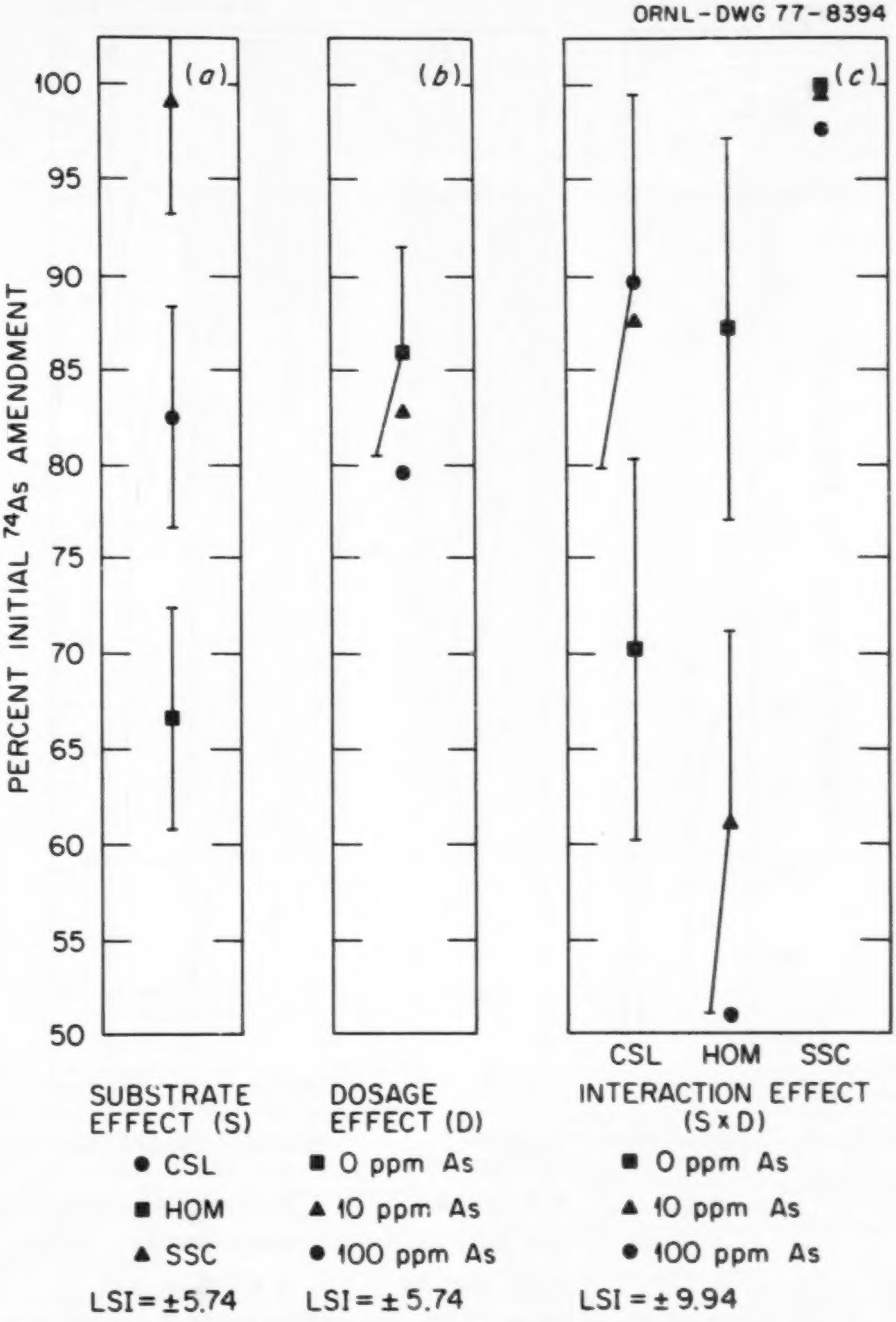

Figure 83. The effects of substrate, As dosage level and their interaction on the percent of the initial ^{74}As amendment retained in the soils of microcosms. Least significant intervals are presented when means are significantly different ($P \leq 0.05$).

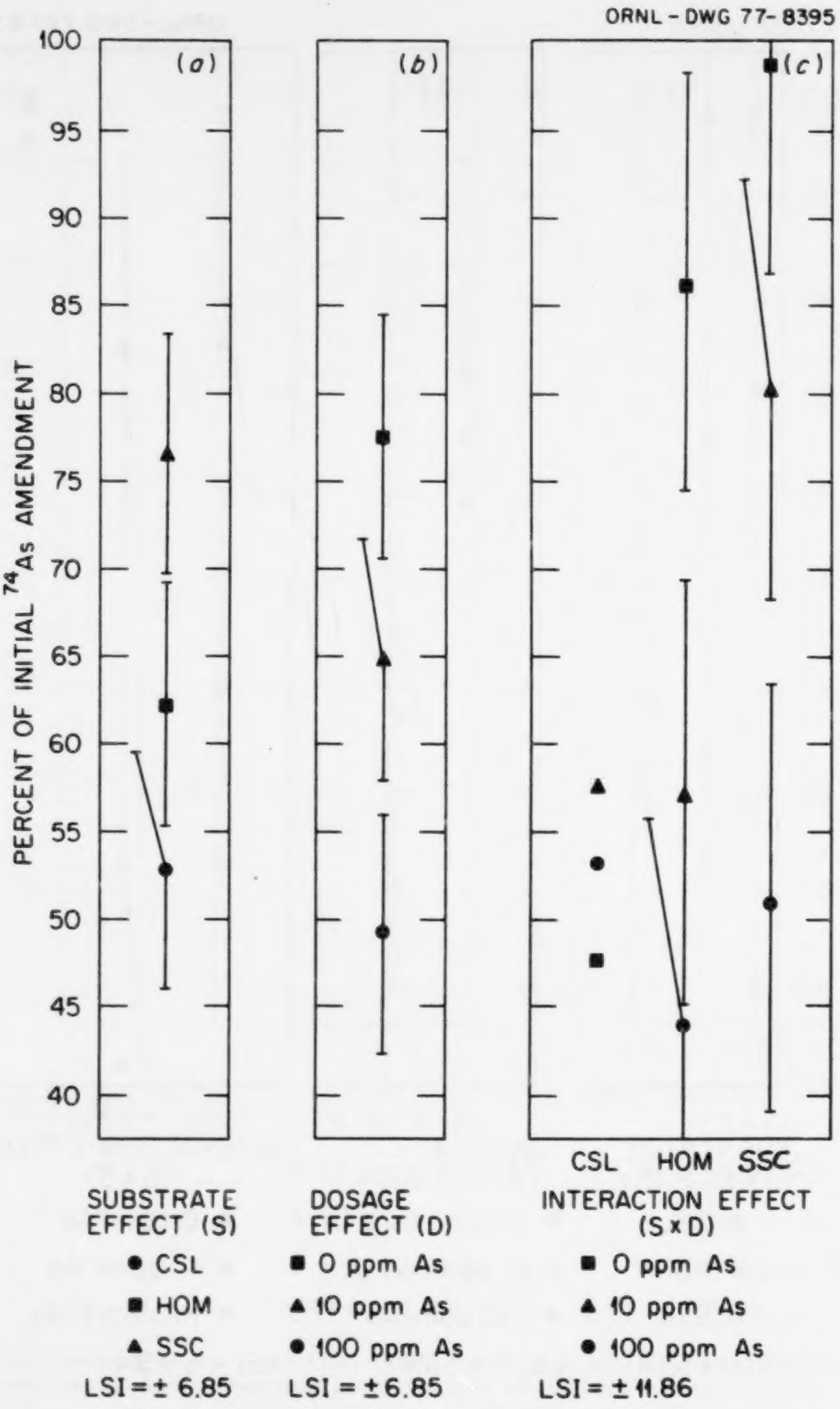

Figure 84. The effects of substrate, As dosage level and their interaction on the percent of the initial ^{74}As amendment retained within the uppermost one cm of microcosm substrates. Least significant intervals are presented when means are significantly different ($P \leq 0.05$).

centimeter. The soil of SSC microcosms retained significantly more ^{74}As than either HOM or CSL microcosms (Figure 84a). Arsenic dosage level resulted in a significant effect ($P < 0.0008$) on ^{74}As retention in the uppermost centimeter of all substrate types. In addition, ^{74}As retention increased with increasing As dosage level (Figure 84b).

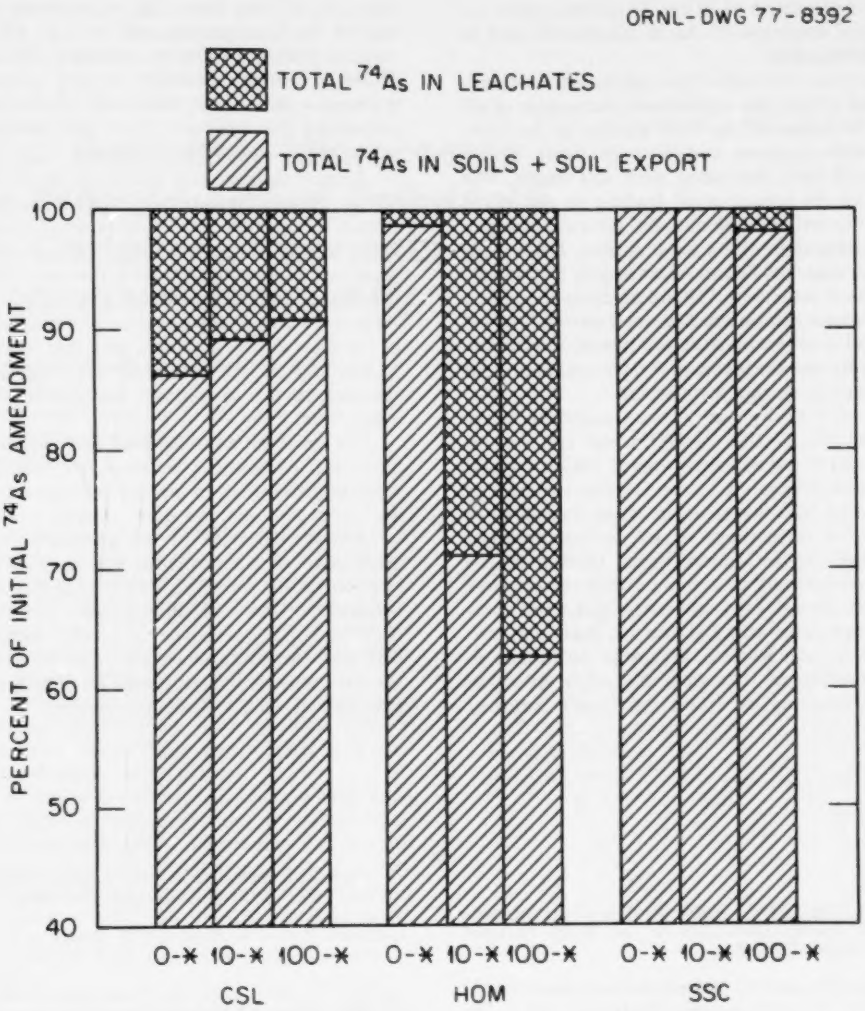

Figure 85. Materials balance for ^{74}As recovered in microcosm components of leachate and soil. 0-* denotes 1 ppm As, 10-* denotes 10 ppm As, and 100-* denotes 100 ppm stable As dosage level.

Substrate type and As dosage level interacted significantly ($P \leq 0.02$) with HOM and SSC microcosms demonstrating decreasing ^{74}As retention with increasing As dosage level.

CO_2 Efflux and Leachate pH Measurement

Carbon dioxide respiration was measured for all microcosms to determine equilibrium after fabrication and to disclose any arsenic dosage effects. Arsenic dosage level had no significant effect on microcosm respiration within the various substrate treatments.

Only CSL microcosms demonstrated an effect of As dosage level on leachate pH. CSL microcosms dosed with 10 and 100 ppm As exhibited significantly higher leachate pH values from day 191 until the conclusion of the experiment.

DISCUSSION

The major microcosm components with respect to ^{74}As behavior in this experiment were leachates and soil. Radioarsenic was lost from microcosms in leachates and retained in microcosms by soil. CSL microcosms were considered to best represent the natural grassland ecosystem because of their relatively greater abiotic and biotic complexity, and their retention of natural soil structure as compared to HOM or SSC microcosms. A materials balance for ^{74}As added to the various microcosm types is shown in Figure 85. CSL microcosms responded differently than both HOM and SSC microcosms with respect to the effect of arsenic dosage level on the amount of ^{74}As in leachates and in soils. The most dramatic dosage effect was observed for HOM microcosms where ^{74}As in leachate increased with increasing As dosage level; and, correspondingly ^{74}As retained by this substrate decreased with increasing As dosage level. SSC microcosms exhibited similar, but less pronounced trends. It should be noted that SSC microcosms dosed with no As or 10 ppm As demonstrated virtually no loss of ^{74}As in leachate, the soil of these microcosms retained most of the amendment. In fact, except for the first leaching, little ^{74}As was transported from any of the substrate types in leachate. Field studies, conducted on intact grassland plots, of ^{74}As transport showed no radioarsenic in soil water collected at a depth of 30 cm. This observation indicated that vertical transport of As in microcosm and in field plots was comparable.

The observation that the bulk of the radioarsenic amendment was retained within the uppermost centimeter of all substrates tested is supported by field studies of As herbicide mobility in soils (Johnson and Hiltbold, 1969). In that study As content of soils decreased with soil depth. The effect of increasing As dosage level leading to decreased ^{74}As retention by the soil of HOM and SSC microcosms may be largely due to saturation of As binding sites. Fassbender (1975) has demonstrated that Fe and Al are largely responsible for the fixation of As in some soils. CSL microcosms showed no such evidence for arsenic saturation. Relatively similar levels of ^{74}As in leachates and in soil occurred at each dosage level tested.

saturation. Relatively similar levels of ^{74}As in leachates and in soil occurred at each dosage level tested.

Soil retention of ^{75}As by the various substrate types followed the order SSC > CSL > HOM and may be explained by differences of the soil structures of SSC and HOM microcosms to that of CSL microcosms. Greater amounts of ^{74}As were retained by SSC microcosms, when compared to CSL microcosms, due to increase in soil surface area entailed in fabricating the SSC microcosms. Mixing of the sand and soil broke up all soil crumbs such that the soil was finely divided. Even though only one-tenth of the soil component of SSC microcosms was Captina silt loam soil, the increase in available soil surface area was sufficient to allow greater adsorption of ^{74}As than the other substrate types. HOM microcosms retained relatively smaller amounts of ^{74}As than CSL microcosms due to the crumb size of the homogenized soil (~ 1.0 - 2.0 mm in diameter) used to fabricate HOM microcosms. The crumb size of the HOM substrate provided a very porous texture with numerous soil water channels. These factors effectively decreased the residence time and adsorption of the ^{74}As amendment in HOM microcosms.

Arsenic dosage level had no effect on the respiration of CO_2 by any of the substrate types. This observation is supported by field studies by Witkamp (unpublished data). In that study no differences in CO_2 efflux were noted for intact grassland plots amended with the same As dosages used in this study. The data presented here indicate that total loss of ^{74}As in leachates may be reliably predicted from the amount of ^{74}As lost at first leaching; and, that total retention of ^{74}As in soil may be determined by assaying the amount of ^{74}As retained in the uppermost centimeter of substrate at the same time.

The relative comparisons and microcosms, based on structural differences between the substrates and used to explain differences in arsenic transport, indicate that intact soil cores provided the most reliable, available information on arsenic behavior in the grassland. While differences were large, each microcosm type used may be viewed more appropriately as simulating a distinct terrestrial soil ecosystem. The CSL microcosms would in this context represent the grassland; the HOM microcosms, a plowed soil; and, the SSC microcosms, a sandy soil. In this context, the data provide valuable predictive information on arsenic behavior in each of these ecosystems.

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The Use of Soil/Litter Microcosms With and Without Added Pollutants To Study Certain Components of The Decomposer Community

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ABSTRACT

Soil/litter microcosms were prepared and maintained in a "life-support" system. Variation of 4 and 11% in carbon dioxide production and oxygen consumption rates, respectively, were observed in 29 microcosms. Populations of bacteria and fungi were found to grow in a "normal" manner with respect to disruption and soil moisture. The greatest respiratory activity was found in the litter.

Metals salts whether considered toxic or nontoxic species at high concentrations may greatly inhibit microcosm respiration, presumably due in part to a salt effect that may mask some toxics effects.

Toxic effects of many metals at realistically high concentrations were observed both singly and in combination. Experiments with chelating agents suggest that soil organic matter may markedly alter toxic substance effects. The toxic effects of certain metals and non-metals may be observed in respiratory changes prior to detectable population changes.

INTRODUCTION

Decomposition of dead organic matter is an essential component in the biological cycle of matter (up to 80-90% primary production is ultimately acted on by decomposer organisms (Odum, 1971; Whittaker, 1970; Witkamp, 1971) in the upper soil layers (Figure 86). It is becoming increasingly apparent that toxic materials from anthropogenic sources are introduced into the soil with real and potential inhibitory effects on the decomposer organisms resulting in toxic effects on both primary producers and their consumers (Tyler, 1975; Lighthart and Bond, 1976; Williams and David, 1973; Rühling and Tyler, 1973).

Soil decomposition may be thought of as a very complex matrix of biological, physical, and chemical process being carried on in a time/space continuum. The system is driven by a set of time/space dependent qualitative and quantitative natural variables such as rainfall, temperature, and nutrient input. In order to detect, understand, and ultimately predict effects of toxic materials on soils as many variables as practicable must be controlled. Further, if toxic materials are to be used, the testing and disposal of these substances must be simple, convenient, and practical. To meet these needs, a soil/litter microcosm contained in a "life-support" system was designed, constructed, and used to observe variables in integrating responses (e.g., community respiration) and differentiated responses (e.g., population quality and quantity).

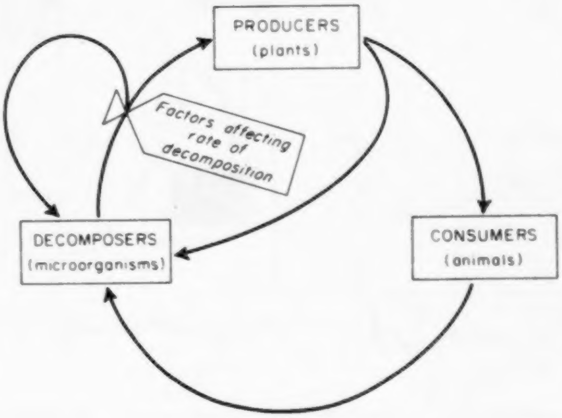

Figure 86. Simplified systems diagram of the organic matter cycle.

The purpose of this report is to present a brief description of a soil/litter microcosm and "life support" system and its use to observe certain natural effects such as; (a) changes in respiration over time as a result of (b) variation between "identically" prepared sets of microcosms, (c) soil/litter moisture level alterations and (d) qualitative and quantitative effects of selected salts. Other evaluations of the microcosm system included its capability to detect, through respiration rate changes, pollutant perturbations caused by (1) high concentrations of various metals and nonmetals, (2) combinations of two toxic substances and (3) the effects of toxic substances on organic matter amended microcosms. The general direction of future research using this microcosm system is also discussed.

METHODS

Criteria followed for the design and development of the microcosm system were: (a) to study soil/litter microbiology, (b) ability to measure biological "intergrater-indicators," (c) ability to measure "differentiated-indicators," (d) operation for extended time periods, (e) simplicity

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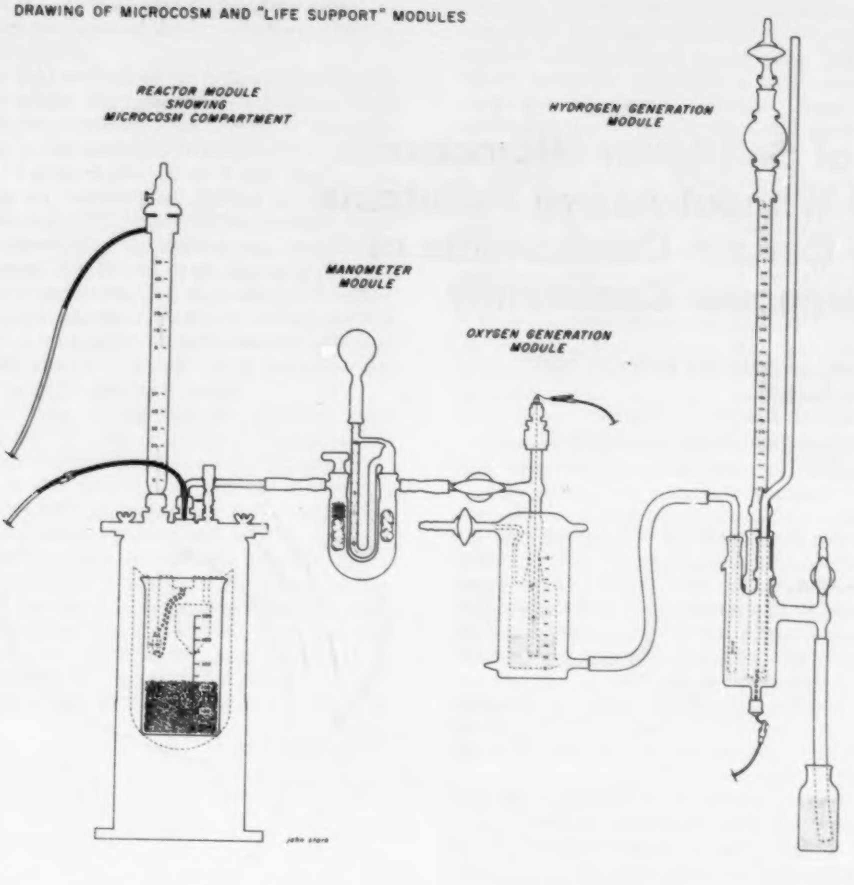

Figure 87. Drawing of microcosm "life support" system including reactor, pressure monitoring, oxygen and hydrogen generating module.

of design and operation and (f) minimization of instrumentation size.

A. "Life-support" system design.

The system consists of a beaker inserted into a "life-support" system (Figure 87). This system consists of (a) reactor chamber, (b) temperature maintenance and heat generation monitoring module, (c) carbon dioxide production monitoring module, (d) pressure monitoring manometer and (e) an oxygen generating module with consumption monitoring capability.

The reactor chamber module is constructed from a one liter Dewar flask large enough to accept the glass microcosm insert (600 ml glass beaker). The flask was sealed with a foam stopper equipped with a small bore glass tube connected to the oxygen generator module, a thermal regulator, and wires to a heating resistor connected to the mercury bulb of the thermal regulator. The thermal regulator and heater were electrically interconnected to maintain the temperature in the reactor at the "set point" of the thermal regulator. Reactor heat loss and resistor heating constants were measured and used to evaluate the heat production in each reactor. Due to technical difficulties, results on biogenic heat production will not be given at this time.

Carbon dioxide within the reactor was trapped in 10.0 ml of 0.6 *N* NaOH. Every 24 or 48 hours during an experiment the NaOH was withdrawn through a permanently placed needle and valve arrangement in the reactor chamber top, and titrated. Data are calculated as milliliters carbon dioxide generated during the trapping period and presented as accumulative milliters carbon dioxide produced over time.

Oxygen consumed during microcosm respiration in the closed system results in reduced internal pressure, activating an electrolytic switch, which causes ocygen to be delivered to the reactor from a hydrolytic oxygen generator similar to the one described by McGarity, et al., (1958). The system described here employs a sodium sulfate/agar salt bridge imbedded in fritted glass located between the hydrogen and oxygen generating electrode. This placement eliminated barometric pressure change effects which could cause unwarranted operation of the electrolytic switch. Ozone or other gases generated during the electrolytic reaction were trapped in activated charcoal and nickel dibutyldithiocarbamate impregnated glass wool located in a U-tube between the oxygen and the reactor chamber (Woodland, 1973). Any gas leaks in the slightly positive pressure system was monitored with a closed water manometer. Soil and litter moisture were determined gravimetrically on 105°C oven-dried samples. Surface and spread plate counts of total hydrotrophic bacteria and micro-fungi were counted in triplicate on plates of 10-fold sterile phosphate buffered dilutions of agitated soil and litter samples on the following media: acidified potato dextrose agar for fungi and Bunt and Rovira's medium for bacteria. Incubation was at room temperature for two weeks.

B. Experimental

Routinely a battery of microcosms were prepared by simultaneously sieving through a 1/16" mesh screen, 150 grams (air dry weight - DW) of homogenized and moistened (60 to 80% of field water holding capacity) soil, into a 600 ml beaker. This soil was compacted uniformly and then covered with 15 grams moistened Douglas Fir tree needle litter previously sieved through a 1/16 inch mesh screen. The litter was also compacted. Except where stated, microcosms were preincubated at 20°C for 10-14 days after preparation before testing was begun (Jenkenson and Poulson, 1976).

Deviation from the foregoing procedures were as follows: (a) microcosm chronology of respiratory and organism changes were observed in microcosms immediately placed in "life-support" systems without preincubation; (b) variation of respiration among 6 sets (totaling 29 microcosms) of similarly prepared and incubated microcosms were observed within like treatments; (c) affects of different weights of soil and litter combinations on microcosms respiration were observed with 0, 35.5, and 75 grams (dry weight) forest soil, overlain with 0, 15, 30 and 45 grams (DW) Douglas Fir tree needle litter; (d) moisture effects were observed on microcosms prepared and maintained at 10, 30, and 60% of field water holding capacity (FWHC); (e) 25 cultured arthropods (*Isotoma* sp.) and 3000 nematodes (*Chepholobus persequnis*) were added to microcosms to observe the effects on respiration and population of animals after 1500 mg Cd Cl$_2$/kg soil-litter (DW) was injected in the standard manner at either 0, 5, or 15 days after animal introduction; (f) to survey the effects of injecting the following mean microcosm concentrations of trace elements on respiration. CdCl$_2$ (1500 mg/kg) CoCl$_2$ (3000), Na$_2$SO$_4$ (1000), SeO$_2$ (680), HgCl$_2$ (30), ZnCl$_2$ (7500), PbCl$_2$ (200), NiCl$_2$ (700), CrCl$_2$ (100), CuCl$_2$ (50), MnCl$_2$ (850), MoO$_3$ (10), CaCl$_2$ (6131), H$_3$BO$_3$ (60), LiCl (100), SnCl$_2$ (10), LaCl$_3$ (100), SbCl$_3$ (10), and AgNO$_3$ (10); (g) to observe the effect of toxicant concentration on microcosm respiration of 0.0, 0.01, 10.0 mg CdCl$_2$/kg (DW) when injected into 3 microcosms; (h) injection of 6.6 mM CdCl$_2$, CuCl$_2$, MgCl$_2$, or CdCl$_2$ + CuCl$_2$; or 10 mg SeO$_2$/kg, 25 mg CdCl/kg or both combined into microcosms to observe single and combined toxicant effects on respiration; (i) injection of 0.1761, 1.761, and 17.61 mM Na$_2$SO$_4$, or 0.132., 1.32 and 13.2 mM of NaCl to observe ion and salt effects on microcosm respiration and finally, (j) injection of 1500 mg of cadmium as Cd humic acid complex/kg (DW) (Aldrich Chemical Company Inc. Milwaukee, Wisconsin) 1500 mgCd-DTPA (diethylenetriamine pentaacetic acid) complex/kg (DW), and the non cadmium complex as controls to observe the effects of organic complexation of a toxic substance on microcosm respiration.

Preparation of the Cd saturated humic acid (HA) complex was accomplished by adding CdCl$_2$ in an aqueous solution of pH 7.0 HA (0.07 gm-HA/ml distilled water) and dialyzing the precipitate against distilled water until Cl$^-$ free while the Cd-DTPA complex was prepared by passing Na-DTPA through a Cd saturated Dowex-50 resin.

The standard injection procedure was to prepare aqueous solutions of the test material to a concentration that would give the desired microcosm concentration when a total of 2 ml was injected into a plane 1 cm from the surface of the litter, and a total of 4 ml into planes 1 and 3 cm from the soil surface. Injection in each plane was at 46 gridded sites.

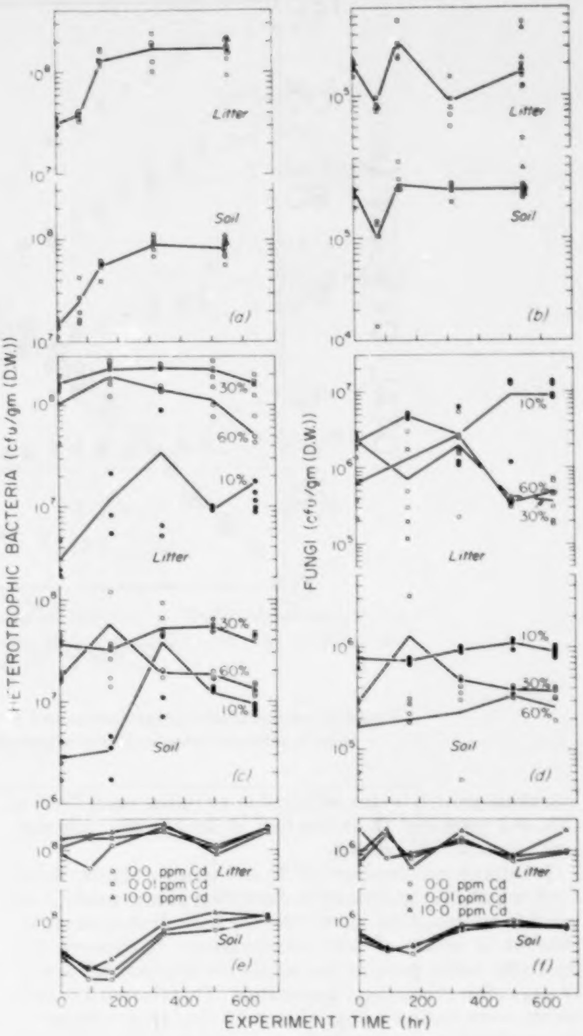

Figure 88. Graphs of heterotrophic bacterial and fungal propogules (CFU=colony forming units) in soil and litter in three "identically" processed microcosms (a&b); three incubated at 10, 30 and 60% of field water holding capacity, respectively (c&d); and three microcosms injected with distilled water 0.0, 0.01 and 10.0 ppm final mean CdCl$_2$ concentration, respectively (e&f).

RESULTS AND DISCUSSION

1. Microcosm Chronology:

Population density of microorganisms particularly the bacteria in the microcosms, changes immediately after microcosm preparation (Figure 88). There is a rapid increase in the first ten days from 10^7 to 10^8 bacteria/gm (DW) of soil or litter. The population remains almost constant at the higher level for at least 20 days longer. This rapid

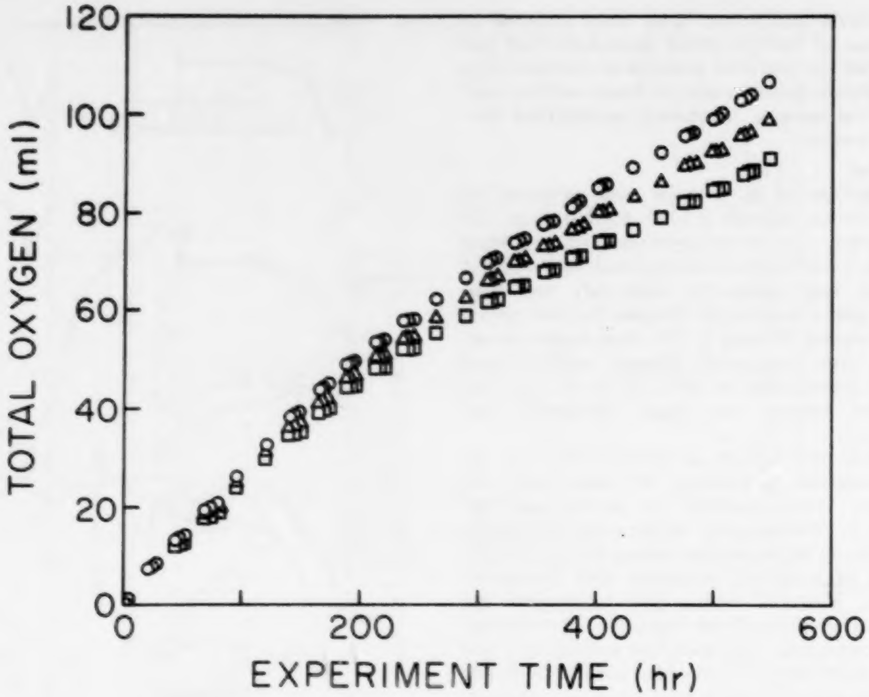

Figure 89. Accumulated oxygen consumed through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms.

microbial growth is also reflected in an initial rapid flush of CO_2 and uptake of O_2 in the first 10 days in the "life-support" systems (Figure 89).

Jenkinson and Poulson (1976) also found a similar initial flush which they attributed to disruption of previously inaccessible microbial nutrients released from destroyed organisms as a result of soil manipulation. It appears that relatively stable populations within the microcosms prevail 10 days after microcosm preparation. Therefore, all experiments were initiated only after a 10 day preincubation of the prepared microcosms. It was reasoned that a 10 day "flush" was an adjustment and unless one's interest was studying the effects of treatments during the adjustment period the microcosms should be allowed to proceed to adjustment "completion" before stressing the systems.

2. Microcosm Variation:

Variation of oxygen consumption and carbon dioxide production within 5 sets of 2 to 8 "identically" prepared and incubated microcosms was 0.54 ± 0.05 ml/hr (C.V. = 10.7 ± 3.7%) and 0.43 ± 0.03 ml/hr (CV = 3.9 ± 1.5%) for 150 gms (DW) soil plus 15 gms (DW) litter (from Table 29). These data indicate a reasonable capability to prepare similarily acting soil systems and would allow detection of treatment effects to a reasonable degree of sensitivity e.g.;

$$P[(-2^{*}\ CV)^{*}\ \begin{matrix}\text{control}\\ \text{microcosm}\\ \text{rate}\end{matrix} \le \begin{matrix}\text{control}\\ \text{microcosm}\\ \text{rate}\end{matrix} \le (2^{*}CV)^{*}\ \begin{matrix}\text{control}\\ \text{microcosm}\\ \text{rate}\end{matrix})$$

$$= 0.95$$

where CV is 10.7% for oxygen and 3.9% for the carbon dioxide rates.

The respiratory quotient for these soil and litter samples which were corrected over several seasons, was 0.80 ± 0.03 indicating that the mean oxidation state of this material was that of a proteinaceous to lipid material (White, Handler and Smith, 1973).

3. Soil and Litter:

The amount of soil and/or litter dramatically effects total respiration (Figure 90). The majority of respiration is due to processes carried on in the litter as opposed to the soil i.e., compare soil of 35 gm with litter of 30 gm. Also, calculations show that the thinner the litter layer the more oxygen consumed per gram of litter per unit time. Thus, one must be careful to prepare microcosms with similar litter volumes and densities as well as chemical characteristics if test treatments are to be compared.

4. Soil Moisture Effects:

The effect of soil moisture on microbial populations is shown in Figure 88, c&d. Bacterial populations seem to prevail under moist conditions and fungi under dryer conditions.

The integrated biological activity as deduced from oxygen consumption and carbon dioxide production (Tabie 29, Figure 91) showed a marked increase at higher soil

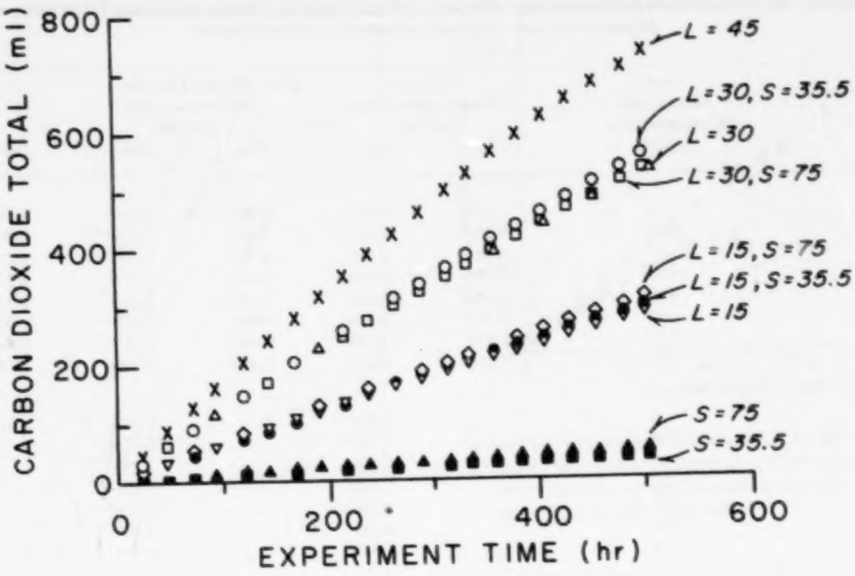

Figure 90. Accumulated sum of carbon dioxide produced in "identically" incubated coniferous forest microcosms containing the indicated combinations of soil (S) and/or litter (L) in grams dry weight.

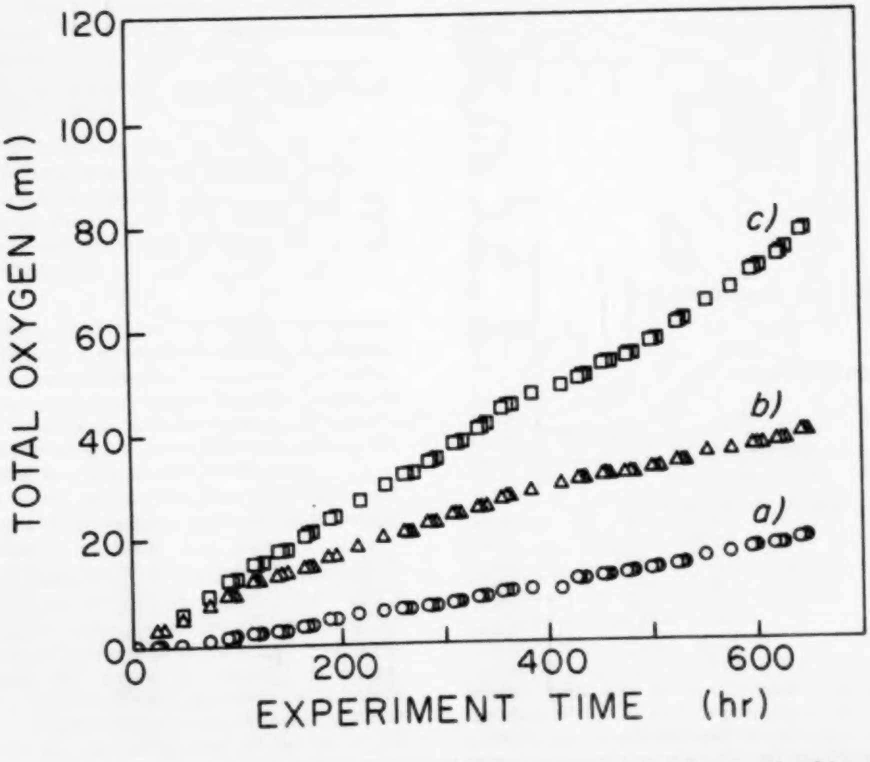

Figure 91. Accumulated oxygen consumed through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms treated to an initial soil moisture of (a) 10%, (b) 30%, (c) 60% of field holding capacity.

Table 29—Tabulation of Respiratory Rates for "Identically" Prepared and Incubated Coniferous Forest Soil/Litter Microcosms Within Each Experiment, and Subjected to the Indicated Treatments.

Microcosm Treatment	Mean Microcosm Concentration (ppm[1])	Oxygen Consumption Rates (ml/hr)		Carbon Dioxide Production Rate (ml/hr)		Respiratory Quotient (CO_2/O_2)	
		Pre-[2]	Post-[2]	Pre-	Post-	Pre-	Post-
Experiment 14							
Control	0	0.508	0.449	0.385	0.361	0.76	0.80
$CdCl_2$	1500	0.499	0.196***	0.397	0.200***	0.80	1.02
$CoCl_2$	3000	0.537	0.416	0.402	0.184***	0.75	0.44
Na_2HAsO_4	1000	0.550	0.526	0.403	0.364	0.73	0.69
SeO_2	680	0.440	0.250***	0.368	0.213***	0.84	0.85
$HgCl_2$	30	0.376	0.345	0.391	0.276***	1.04	0.80
$ZnCl_2$	7500	0.432	0.148***	0.404	0.109***	0.94	0.74
$PbCl_2$	200	0.518	0.414	0.413	0.348	0.78	0.84
Experiment 16							
Control	0	....	0.484	....	0.356	....	0.74
$NiCl_2$	700	....	0.250***	....	0.174***	....	0.70
$CrCl_2$	100	....	0.385	....	0.255***	....	0.66
$CuCl_2$	50	....	0.380	....	0.292***	....	0.77
$MnCl_2$	850	....	0.252***	....	0.206***	....	0.82
$CdCl_2$	1500	....	0.217***	....	0.165***	....	0.76
MoO_4	10	....	0.540	....	0.414	....	0.77
Experiment 22							
Control	0	0.585	0.510	0.498	0.397	0.77	0.78
$CaCl_2$	6131	0.591	0.235***	0.472	0.164***	0.80	0.70
V O_5	60	0.611	0.481	0.469	0.379***	0.77	0.79
LiCl	100	0.581	0.331**	0.472	0.264***	0.81	0.80
$SnCl_2$	10	0.573	0.503	0.438	0.385	0.77	0.77
$LaCl_3$	100	0.545	0.425	0.468	0.355***	0.86	0.83
$SbCl_3$	10	0.532	0.457	0.459	0.400	0.86	0.88
$AgNO_3$	10	0.683	0.606	0.506	0.442***	0.74	0.73
Experiment 13[3]							
Control 1	....	0.568	....	0.407	....	0.717	....
Control 3	....	0.559	....	0.417	....	0.746	....
Control 4	....	0.662	....	0.418	....	0.633	....
Control 5	....	0.517	....	0.404	....	0.781	....
Control 6	....	0.447	....	0.402	....	0.900	....
Control 7	....	0.473	....	0.416	....	0.879	....
Control 8	....	0.537	....	0.407	....	0.758	....
Experiment 3							
Moisture	10%	0.014	....	0.028	....	1.10	....
Moisture	30%	0.050	....	0.056	....	0.76	....
Moisture	60%	0.122	....	0.091	....	0.75	....
Experiment 18a[3] Long term variation (900 hrs)							
Microcosm 5	....	0.455	....	0.377	....	0.83	....
Microcosm 6	....	0.460	....	0.395	....	0.86	....
Experiment 18b Short term variation							
Microcosm 5	....	0.508	....	0.438	....	0.86	....
Microcosm 6	....	0.459	....	0.421	....	0.92	....
Microcosm 7	....	0.616	....	0.448	....	0.73	....
Microcosm 8	....	0.627	....	0.489	....	0.78	....
Experiment 24							
Na_2SO_4	0.1761 mM	0.430	0.417	0.408	0.338	0.95	0.81
Na_2SO_4	1.761 mM	0.459	0.361	0.414	0.282	0.90	0.78
Na_2SO_4	17.61 mM	0.534	0.308	0.446	0.235	0.84	0.76
NaCl	0.132 mM	0.516	0.444	0.401	0.347	0.78	0.78
NaCl	1.32 mM	0.404	0.229	0.386	0.199	0.96	0.87
NaCl	mM	0.430	0.185	0.382	0.145	0.89	0.78
LiCl	552.0 ppm	0.481	0.319	0.470	0.293	0.98	0.92
H_2O	....	0.540	0.473	0.422	0.357	0.78	0.76
	Mean	0.474	0.342	0.416	0.275		
	Variance	0.052	0.102	0.030	0.076		
	Coe. of Variation (%)	11.0	29.8	7.2	27.7		

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[1] As metal or non metal

[2] Pre- and Post-pollutant treatment

[3] Rate calculated from entire experimental time.

** > 2 Coefficients of variation from experiment control.

*** $\geq$ 3 Coefficients of variation from experiment control.

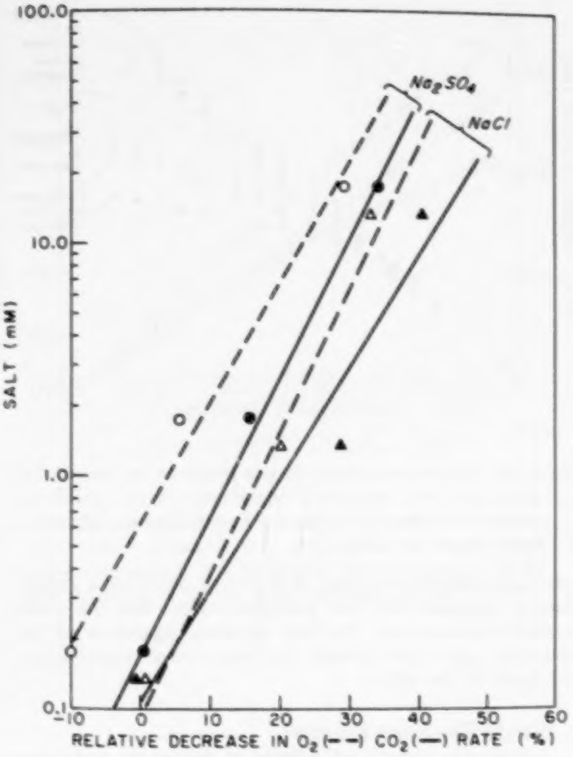

Figure 92. Salts (NaCl, Na_2SO_4) effects (compared to initial control rate) on respiration in "identically" prepared coniferous soil/litter microcosms.

moistures, i.e., respiration was two to three times faster at 60% than at 10% soil moisture.

The RQ's at 30% and 60% soil moisture indicates a "usual" oxidizing process compared to other results in Table 29, but at 10% moisture a more oxidizable substrate may be metabolized. These observations may be correlated with the relatively high fungal activity at low soil moisture and their sacchrolytic reputation.

5. Salt Effects:

As seen in Figure 92 (and Table 29), the quality and increasing quantity of salts may markedly inhibit respiration in the coniferous forest soil/litter microcosms. The anion, chloride, appears to have a more toxic effect than sulfate on a molar basis, but on an ion basis the inhibitory differences would be nil. Thus there is an ion effect component that should be recognized as a part of any toxic effect.

The salt or ion effect did not alter metabolic processes significantly in terms of the oxygen consumption to carbon dioxide production ratio (i.e., RQ).

6. Biological Age and Effect of Toxicant Addition:

When microcosms are treated with 1500 ppm Cd^{++} at 0, 5 and 20 days incubation [illegible] preparation O_2 consumption and CO_2 production i[illegible]uced 22-30-30% and 38-43-35%, respectively (Figure [illegible]ased on the RQ data (unpublished) which rema[illegible]atively constant at 0.71 to 0.75 there is no obvious relationship between biological age and respiration. Because no regular pattern in the microbial population stress effect with microcosm age has been detected, we assume that the time of stress on microcosm respiratory activity is relatively small.

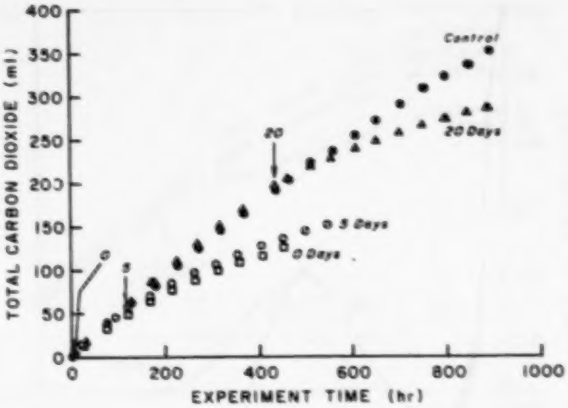

Figure 93. Accumulated carbon dioxide produced through time from "identically" prepared and incubated coniferous forest soil/litter microcosms treated to 1500 ppm $CdCl_2$ at either 0, 5 or 20 days of biological age.

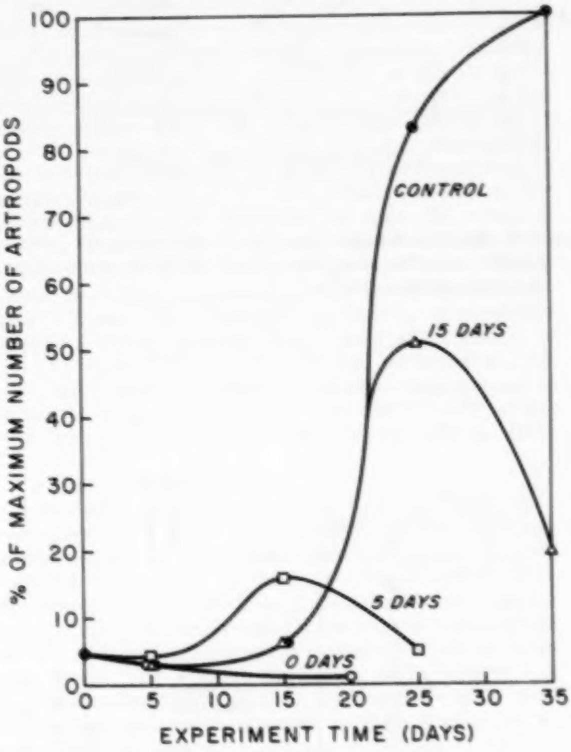

Figure 94. Survival of 0, 5 and 15 day old Collembola populations in "identically" prepared coniferous forest soil/litter microcosms treated to 1500 ppm $CdCl_2$.

For 25 adult Collembola (*Isotoma* sp.), and approximately 3,000 free living nematodes added to microcosms, the time of $CdCl_2$ addition was critical. That is, pollutant added simultaneously with organisms (zero day experiment) or within one week (5 day experiment) show little or no growth of Collembola and a 98% decrease in nematodes after 20 to 25 days incubation (Figures 94 and 95). Later

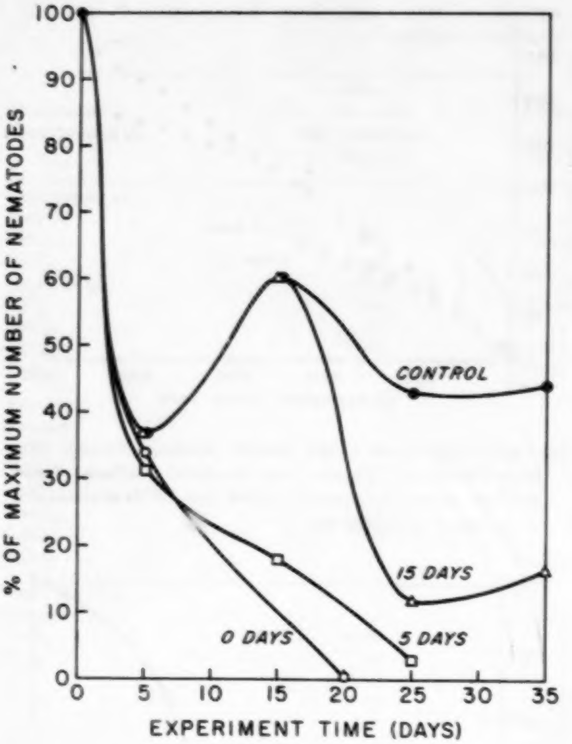

Figure 95. Survival of nematodes in 0, 5, and 15 day old "identically" prepared coniferous forest soil/litter microcosms treated to 1500 ppm $CdCl_2$.

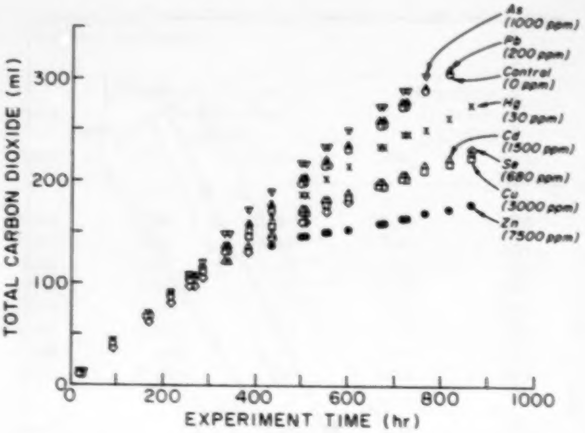

Figure 96. Accumulated carbon dioxide produced in "identically" prepared and incubated coniferous forest soil/litter microcosms treated to a final mean concentration of various heavy metals (as salts).

pollutant additions resulted in the same population reductions. It appears that this pollutant effect has the same general characteristics for both animals regardless of the biological age of the system; i.e., cessation of reproduction and death of the adults.

7. Single Toxicant Effects:

Statistically significant effects of particular trace elements added as single salts to the microcosms showed an inhibition of CO_2 production by Cu, Hg, Or, Co, V, and La while As showed a stimulation, whereas both CO_2 production and O_2 consumption were inhibited by Cd, Se, Zn, Ni, Mn and Li (Table 29, Figures 96-97). It is possible that oxygen consumption is inhibited for the former group but the analytical methods do not detect the change. It does not appear that the statistical method used to limit significance in this case causes the result because RQ's appear to be logical

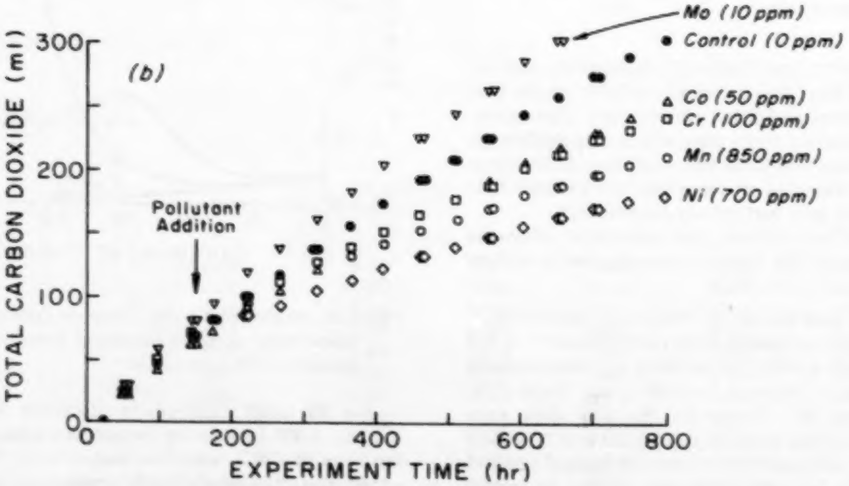

Figure 97. Accumulated CO_2 produced through time in "identically" prepared and incubated microcosms treated with the indicated trace elements.

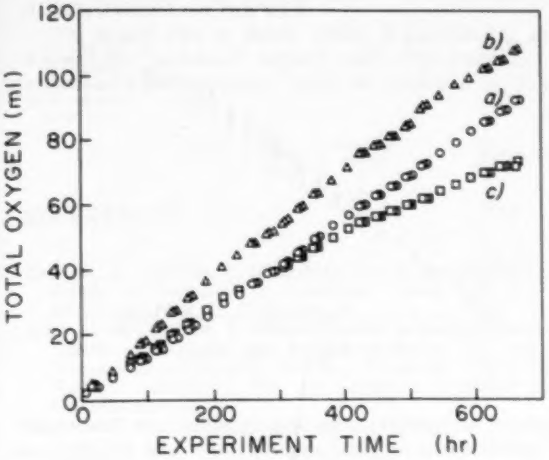

Figure 98. Accumulated oxygen consumed through time in three "identically" prepared and incubated coniferous forest microcosms treated to a final mean concentration of (a) 0.0 ppm, (b) 0.01 ppm, (c) 10.0 ppm cadmium chloride.

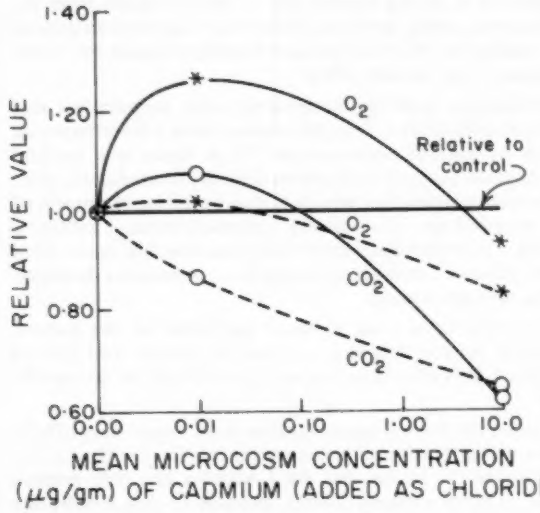

Figure 99. Graph of the relative oxygen consumption (*), and carbon dioxide generation (0) rate values for the initial 200 (solid lines), and subsequent (dashed lines) hours after cadmium addition (mean microcosm concentration) to Douglas fir soil/litter microcosms.

i.e., 0.7 to 0.8. Further, none of the RQ's except the experiment with Cd and Co are outside two coefficients of variation (Table 29) from experimental controls.

Other results indicate possible additional inhibitory effects by Cr, Hg and Co on oxygen consumption, and stimulation of oxygen consumption and carbon dioxide production by As and Mo. Copper shows both initial stimulation after pollutant addition and subsequently an inhibition of oxygen consumption.

As seen by reduced respiratory activity, it appears that trace elements found in realistic levels can and probably do inhibit soil decomposition processes.

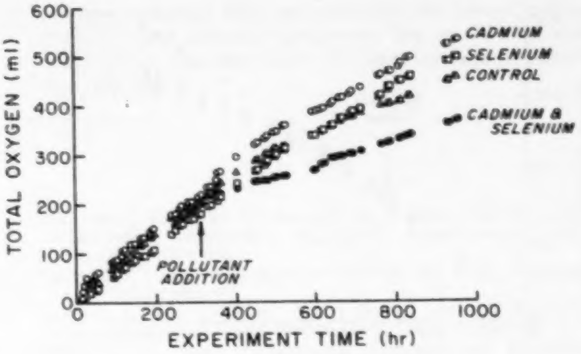

Figure 100. Accumulated oxygen consumed through time in four coniferous forest microcosms treated to a final mean concentration of 0.0 ppm $CdCl_2$ or SeO_2, 25 ppm $CdCl_2$, 10 ppm SeO_2, and both.

Even though microbial density (Figure 88, e & f) is not a detectable response to variation in concentration of added pollutant, respiration (Table 29, Figure 98) varied with the amount of Cd added and with time of observation. At low Cd concentrations (0.01 ppm $CdCl_2$), a transient stimulatory effect (increased respiratory rate) was noted for the initial 200 hours after amendment, followed by recovery to control rates. Respiration at high Cd concentrations (10 ppm) equal that of the control for approximately 300 hours after amendment; thereafter, respiration decreased by 40% compared to the control (Bond et al., 1975; 1976). The mechanism of the dramatic reduction of respiration at high Cd levels is unknown. There was no decrease in numbers of culturable soil or litter fungi or bacteria, although there was a relative increase in oxygen consumption over carbon dioxide production (Figure 99) suggesting uncoupling of oxidative phosphorylation, a common heavy metal effect (White et *al.*, 1973). Stimulation at low levels and inhibition at high heavy metal levels is a common microbial response and is referred to as an oligodynamic action (Salle, 1973) or the Arndt-Schuldtz Law (Lamanna and Mallette, 1965, p. 1897).

8. Toxicant Combinations:

In the Se/Cd experiment as in the high Cd level experiment, there was a delay of several hundred hours before an inhibitory effect on the oxygen consumption was observed (Table 29, Figure 100). In the Se treatment, effects were seen almost immediately after pollutant addition. The interaction effect of the Se and Cd on oxygen consumption rate was 55% greater than when either of the metals were added singly. The reduced respiration in the control was due to the inadvertant deletion of distilled water at the time of metal addition. Previous results indicate respiration remains relatively constant when water is added to the control. These observations indicate that combinations of elements may have unexpected severe effects on soil decomposer processes!

Additionally at higher concentrations (6.6 mM) Cd, Cu, Cd + Cu or Mg there are similar reductions in CO_2 production,rates (Figure 101), indicating that at high concentrations of toxicant, a salt effect may dominate the combined toxic metal effects.

9. Organic Complexation of a Toxic Substance:

Amendment of a coniferous forest soil/litter microcosm with 1500 or 150 mg Cd^{++} as $CdCl_2$/kg caused a statistically

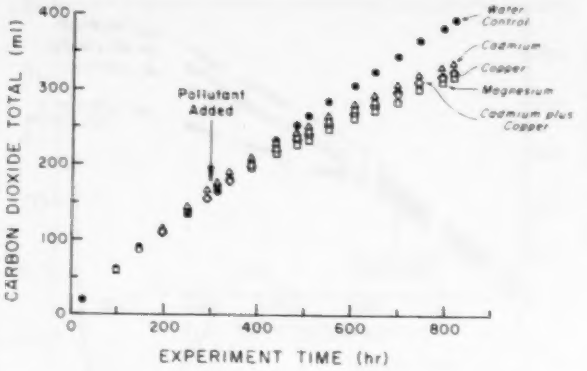

Figure 101. Accumulated carbon dioxide produced through time in "identically" prepared coniferous forest microcosms treated to 6.665 mmoles of magnesium, copper, cadmium or equal amounts of copper plus cadmium as sulfates.

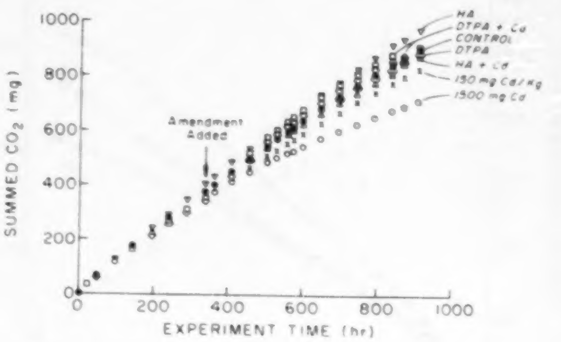

Figure 102. Accumulated carbon dioxide produced in "Identically" treated coniferous forest soil/litter microcosms amended with 1500 mg cadmium/Kg (mean microcosm concentration) free, and chelated with DTPA and humic acid (HA).

significant (P greater than equal to 0.01) reduction in respiration of 38 and 17% of the control respectively (Figure 102), whereas 1500 mg Cd/kg complexed with DTPA ($k_{sp} = 10^{-23}$) was within 3% of the control, not a significant difference. Humic acid (HA) complexed with 1500 mg Cd/kg reduced respiration by 11%, intermediate between DTPA and no complexing agent.

It is apparent from this experiment that the type of organic material probably has a significant effect on the free Cd ion in the soil. Further, the binding of toxic substances by soil components must be an extremely important factor for organism survival. Doyles et al., (1975) found that only 5 $\mu g/Cd^{++}/ml$ of culture fluid caused reduced growth of several species of bacteria. Measurement of free Cd ion in the same culture fluid by Lighthart (unpublished) showed Cd ion values of 0.5 μg Cd^{++}/ml. Assuming soil to have a bulk density of 1.5 gms/ml and free Cd ion concentrations of 0.75 mg Cd^{++}/kg, we would expect to see toxic effects from 150 to 1500 mg Cd^{++}/kg soil.

INTERPRETATION

Pollutant materials research in microcosms appears to be an acceptable alternative in certain instances to study contamination of natural sites. From a statistical point of view the so called "integrater-indicators" of respiration, oxygen consumption and particularly carbon dioxide production, allowed detection of treatment effects in microcosms with reasonable precision, e.g., 10.7 and 3.9% coefficients of variation, respectively. Even the "differentiated indicators" such as organism populations, show reasonable density similarity through time from microcosms up to six weeks old. Numerical abundance may not show the concurrent toxic effect, however.

Microcosms treated to natural variables respond respirometrically and organismally similar to what is thought to occur in nature. The effect of moisture reduction from 60 to 10% of field water holding capacity both reduces respiration and shifts the microcosms population from the less xerophylic bacteria to the more xerophylic fungi. The dryer conditions also select for a more sacchrolytic-microflora.

Replication of microcosms is dependent upon careful attention paid to microcosm preparation. As might be expected, increased salt treatment within the microcosm alters respiration. Both type and quantity of anion effect respiration. On a weight basis NaCl is more toxic than Na_2SO_4 whereas on a ion basis they appear to have equal inhibitory effects. This effect appears to increase logarithmically from at least 18 to 24 mg salt/kg soil + litter. Results from the microcosm system indicates that at high salt concentrations any inhibitory effects of a toxic substance could be "overshadowed" by the salt effect.

Microcosm activity immediately after preparation also approximates what is thought to occur after a disturbance in nature by natural phenomenon. Thus, there is a marked flux of gases (oxygen and carbon dioxide) immediately after microcosm preparation which is due to the rapid growth of the populations, particularly microorganisms, utilizing nutrients liberated from previously inaccessible micro-sites in the soil and nutrients liberated from organisms damaged during soil processing.

The effects of trace element pollution on the natural soil/litter microcosms is a function of amount and type of pollutant. Salt effects on respiration is likely to be significant.

Generally, trace element studies show respiratory effects at concentrations found in nature for Cd, Cu, Se, Hg, Zn, Ni, Cr, Co, Mg, V, Li, La, and As. Cadmium, the only element tested so far at different levels, showed an Arndt-Schuldtz law effect in that stimulation of respiration occurred at low concentration with inhibition at high concentrations.

Combinations such as Cd and Se show greater than additive inhibition of respiration when compared to the individual elements. The mechanism for the toxic effects of these substances are largely unknown but it does appear that organic materials could and probably do decrease the concentration of the ionic forms of some metal species with a resultant decrease in toxic action.

Future research will continue to focus on the survey of soil pollutants using the microcosms. In order to identify specific soil system mechanisms an artificial soil with known biological and chemical constituents is to be prepared and eventually used in the microcosm test system. The test system will also act as the prototype for predictive computer simulation models that will be used to test for differences between predicted and observed microcosm treatment results.

ACKNOWLEDGEMENTS

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The Accuracy of Screening Techniques

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ABSTRACT

A goal of microcosm research is to evaluate the transport, fate, and effects of potentially hazardous substances upon terrestrial ecosystems within confined experimental conditions. The applicability of microcosm results hinges upon their demonstrated replicability, reproducibility, and accuracy. Results must be rapid, cost-effective, and quality controlled. A variety of microcosm types, having intact soil, homogenized soil and artificial substrates, have been tested. The objectives of this paper are to review: (1) parameters which can be nondestructively monitored in microcosms to assess the transport rates of a substance and concomitantly its effects upon ecosystem processes; (2) criteria for and comparison of replicability and reproducibility among microcosm types tested; and (3) criteria for and comparison of the accuracy of results among microcosm types tested. Major conclusions include: (1) carbon (gaseous and dissolved) and macronutrient efflux from systems increases in response to chemical stress; (2) intact soil microcosm units are more replicable and reproducible than others tested; and (3) accuracy of intact soil microcosm type in screening potentially hazardous substances.

INTRODUCTION

Need

Microcosm experimental units are confined, replicable tools which have been developed in response to recent legislative requirements contained within the Toxic Substances Control Act (P.L. 94-469) and the Resource Conservation and Recovery Act (P.L. 94-580). These Acts require testing of potentially hazardous substances resulting from manufacturing, processing, and waste disposal. Criteria for testing apply to those substances which (1) may present an unreasonable risk to health or environment, (2) insufficient data exist for assessment of risk to be made, and (3) testing is needed to develop data needed for assessment of risk. Substances deemed potentially hazardous to health or environment, or produced in substantial quantities (thereby increasing the risk of exposure), can be required to be tested.

The criteria for testing remain unclear (P.L. 94-469) and must be approved by the Environmental Protection Agency (EPA) before use. Procedures for use as the initial screening, termed Level 1 Environmental Assessment: Biological Tests (Duke et al., 1977), have three goals. These are: (1) provision of preliminary environmental assessment data; (2) identification of problem areas; and (3) collation of data needed to prioritorize further assessment activities. Two additional levels of assessment will be developed. Level 2 studies will allow more specific studies investigating rates of transport and levels of effects. Level 3 studies will be designed to discern the exact mechanisms disrupted by the substances, allowing appropriate management control or elimination of problems.

Under legislation, research to develop these screening tools can be sponsored by EPA. Specific testing of the substances, however, are the responsibility of industry. The initial screening reports accepted by EPA are expected to be the 'pacesetters' for following reports. EPA will require only enough data to reach regulatory decisions and will update acceptable screening procedures as the science (or art) progresses. These updates will not jeopardize the acceptability of the earlier-prescribed screening procedures (P.L. 94-469).

Microcosm Screening Techniques

Microcosms have been used to address several levels of biological complexity, including cellular, tissue, organ, organismal, population, community, and ecosystem (Witherspoon et al., 1976). The gamut of experimental units, then, includes pure cultures to excised, intact portions of terrestrial landscapes (Table 30).

Microcosm studies are being used as experimental tools to assess chemical transport, fate, and effects upon

Table 31—Microcosm Test Systems, Substances Tested, and Citations

Microcosm Test System	Contaminant	Citation
Soil cores, no plants	Cd	Ausmus and O'Neill, submitted
	Pu, Cu, Cd, Zn	O'Neill et al., in press
	As	O'Neill et al., in press Jackson et al., in press
	HCB	In experimentation
Soil core with plants	Cd	Van Voris, in preparation
	Pu, Cu, Cd, Zn	Jackson et al., submitted
	As	In experimentation
Watershed, field	Pu, Cu, Cd, Zn	Jackson and Watson, in press Watson et al., 1976

Table 30—Uses That Various Microcosm Units Have for Specific Screening Applications

	Microcosm Type				
Screening Application	Bioassay	Biomagnification	Strata Cores	Excised Systems	In Situ Studies
Total Transport				X	X
Total Fate					
Distribution				X	X
Accumulation				X	X
Strata Transport			X	X	X
Accumulation Within Strata			X	X	X
Trophic Transfer		X	X	X	X
Target Species	X	X	X	X	X
Target Communities		X	X	X	X
Process Disruption			X	X	X
Process Sensitivity			X	X	X
System Disruption				X	X
System Sensitivity				X	X
Mechanisms of Disruption	X	X	X	X	

ecosystem components, specifically populations, and trophic web members (Metcalf et al., 1973; Booth et al., 1973; Focht and Alexander, 1970; Francis et al., 1975). Within these studies, a variety of artificial and homogenized substrates have been used to construct experimental units (Witherspoon et al., 1976). However, these studies address ecosystem components rather than ecosystem processes, such as nutrient cycling and carbon metabolism. The effects upon an ecosystem's ability to compensate for and to recover from chemical stress cannot be inferred from studies of ecosystem components since the interconnections among components to form an ecosystem are not additive (O'Neill et al., 1975; O'Neill et al., in press).

The criteria for microcosm acceptance as screening tools include their demonstrated replicability, reproducibility, and accuracy or realism of the data produced. These will provide initial quality control for screening procedures, and quantitative background information for regulatory decisions.

Witherspoon et al. (1976) discuss the complexities, applications, measured parameters, reproducibility and realism in their review of microcosm research. They summarized the suitability of various designs for assessment of chemical accumulation sites, mutualistic and antagonistic processes, transport of the substances, and identification of critical problems in microcosm use. Problems listed included: (1) lack of demonstrated applicability of transfer rates and distribution of substances within microcosms to ecosystems; (2) lack of comparability or reproducibility of microcosm experiments, and (3) lack of estimated confidence intervals around microcosm measurements of several parameters.

Objectives

We have examined several types of microcosm experimental units with the ultimate purpose of assessing their utility as analogs of terrestrial ecosystem processes. We had three research goals; (1) to construct terrestrial microcosms which can be quantitatively equilibrated, replicated, and reproduced; (2) to identify and nondestructively measure parameters in microcosms which are indicative of ecosystem response to and recovery from stress; and (3) to quantitatively relate microcosm results to ecosystems (Table 31).

There are three objectives of this paper. These are to review: (1) parameters which can be nondestructively monitored in microcosms to assess the transport rates of a substance and concomitantly its effect upon ecosystem processes; (2) the criteria for and compare the replicability and reproducibility among microcosm types tested; and (3) the criteria for and compare the accuracy of results among the microcosm types tested.

MEASURED PARAMETERS

Criteria

There are five criteria which should be satisfied by the parameters chosen to measure in microcosm screening of potentially hazardous substances (Figure 103). First, the parameters measure should be indicative of terrestrial ecosystem function. That is, the measurements should be holistic rather than specific to ecosystem components, such as suspected target populations. Second, parameters should be used which can be nondestructively monitored throughout the experiment. This will allow detection of changes in parameters through time without the confounding which results from repeated removal of components of the microcosm units. Third, parameters monitored should allow rapid data acquisition at several intervals during the experiment. Daily or weekly measurements of parameters in experimental and control microcosm will increase statistical rigor of the screening experiment. Fourth, parameters should be chosen which allow both transport of the tested substance and effects on ecosystem processes to be simultaneously determined. Concentrations of dissolved organic carbon (DOC) and of the substance under study, for example, could both be monitored in leachates periodically taken from microcosms. Finally, parameters which are needed but which cannot be monitored can be determined on replicate microcosms sacrificed at time zero, and on all

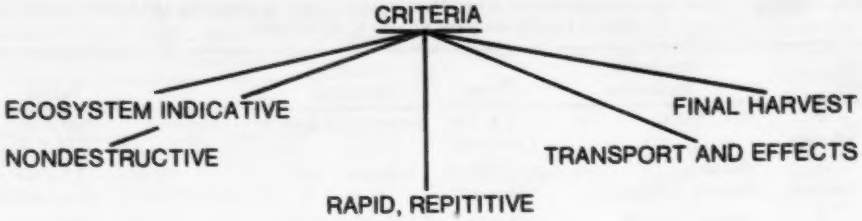
CRITERIA
ECOSYSTEM INDICATIVE
FINAL HARVEST
NONDESTRUCTIVE
TRANSPORT AND EFFECTS
RAPID, REPITITIVE

Figure 103. There are five criteria for parameters to monitor and measure in microcosms.

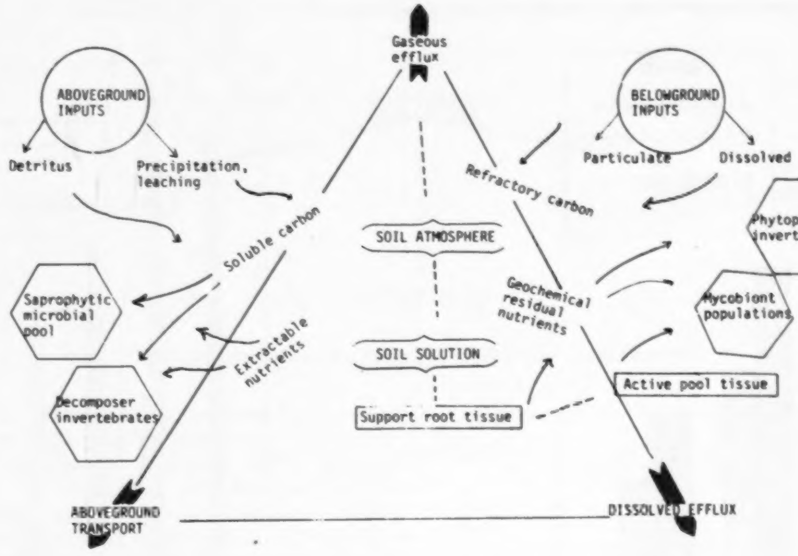
Gaseous efflux
ABOVEGROUND INPUTS
Detritus
Precipitation, leaching
BELOWGROUND INPUTS
Particulate
Dissolved
Refractory carbon
Soluble carbon
SOIL ATMOSPHERE
Phytophageous invertebrates
Saprophytic microbial pool
Geochemical residual nutrients
Mycobiont populations
Extractable nutrients
SOIL SOLUTION
Active pool tissue
Decomposer invertebrates
Support root tissue
ABOVEGROUND TRANSPORT
DISSOLVED EFFLUX

Figure 104. Conceptualization of the interactions occurring within the soil of ecosystems under no stress.

microcosms at time final. These include distribution of tested substance among ecosystem components and estimation of microbial, invertebrate, and plant biomass.

Monitored Parameters: Indices of Ecosystem Response to Stress

A natural candidate for terrestrial monitoring would be the rate at which macronutrients and carbon are exported from microcosms. Shugart et al. (1976) suggest that dissolved nutrients within soil water may be indicative of total ecosystem cycling of calcium. Recent microcosm studies, which treat these experimental units as model ecosystems, have shown that carbon and nutrient efflux from these systems are affected by chemical stress (O'Neill et al., in press). Soil, because it contains the largest number of interacting components within terrestrial ecosystems, becomes the subsystem focused upon to provide initial screening procedures. Because of the large number of interacting components in soil (Figure 104), detrimental effects are exhibited as *increases* in gaseous and dissolved carbon loss, and in increased dissolved macronutrient loss. This loss can be detected irrespective of the specific organisms and enzymatic pathways which were disrupted by chemical stress. The exact mode of effects may not be intuitively perceived. Therefore, focus upon a holistic index of ecosystem response to such an effluent would be desirable. Three studies, using soil only, intact soil-tree sapling units, and a watershed illustrate this point (Table 32). In each case disruption of nutrient cycling processes were detected prior to, or under less stress, than changes in population or community parameters. A recent aquatic microcosm study (Strange, 1976) showed similar increased nutrient mobility in response to herbicide treatment.

Monitoring nutrient loss rates may not be necessary to detect ecosystem response to chemical stress. An index of nutrient status of soil under stressed conditions shows depletion of nutrient pools (Jackson and Hall, submitted; Jackson et al., submitted; Ausmus et al., submitted). Figure 105 illustrates one such depletion of soil carbon from heavy metal stress.

Measurements at Harvest: Distribution and Fate

While the rate of nutrient efflux and of tested substance efflux can be monitored throughout microcosm experiments, the distribution of the tested substance through microcosm components must be determined at the experiment's conclusion. Coupling of monitored data with those at harvest, researchers can determine the mass balance of the tested substance and of the macronutrients of interest. The distribution of lead in forest microcosm components at the end of a 20-month experiment is shown in Figure 106.

Table 32—Population and System Level Parameters Measured in Soil Microcosms, Forest Microcosms, and Forest Under Control and Chemical Stress Conditions (after O'Neill et al. in Press).

Level Control and Treatment		Population Parameter	Values	System Parameter	Values
Arsenic (Na_2HAsO_4)	0 100 ppm	ATP (ppm)	1.58 ± 0.33 1.52 ± 0.30	Leached Ca (μg)	560 ± 36 714 ± 50
Arsenic (Na_2HAsO_4)	0 100 ppm	Bacterial density (10^8/g)	2.75 ± 0.13 3.18 ± 0.41	Leached P (μg)	2.8 ± 0.5 21.0 ± 6.0
Heavy metals	0 11 mg Pb/cm²	Annual branch growth (cm)	347 ± 43 346 ± 68	Leached Ca (ppm)	6.6 ± 0.4 10.0 ± 0.8
Distance from lead smelter	21 km 2.0 km	Litter invertebrate diversity (Shannon Index)	2.3 ± 0.15 2.5 ± 0.23	0.2 litter mass (g/m²)	1008 ± 110 1595 ± 171

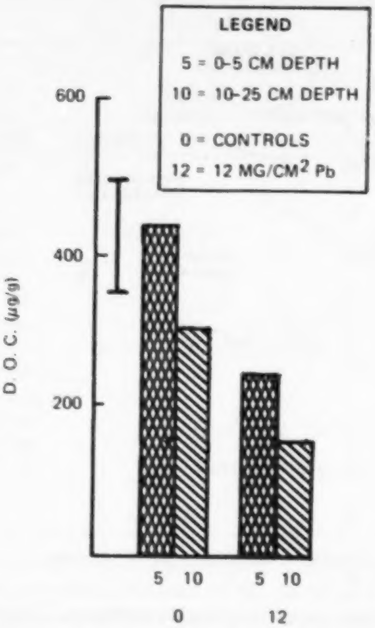

Figure 105. DOC extractable from soil at two depths in forest microcosms as a function of heavy metal impaction (Jackson and Hall, submitted).

Measurements at Harvest: Biological Composition

Detection of hazardous substance effect upon biota can be made at experiment conclusion, also. Biomass differences between treated and control microcosms may indicate population shifts or community degradation due to chemical stress. Table 33 shows three indices of soil microbial populations measured at the conclusion of an experiment. Control microcosms had significantly greater fungal populations than microcosms which had been contaminated with a complex effluent of Pb, Zn, Cd, and Cu (Ausmus et al., submitted).

REPLICABILITY, REPRODUCIBILITY

Criteria

In order to establish quality control of screening procedures, three criteria must be met (Figure 107.). First, the parameters must be predictable through the experimental

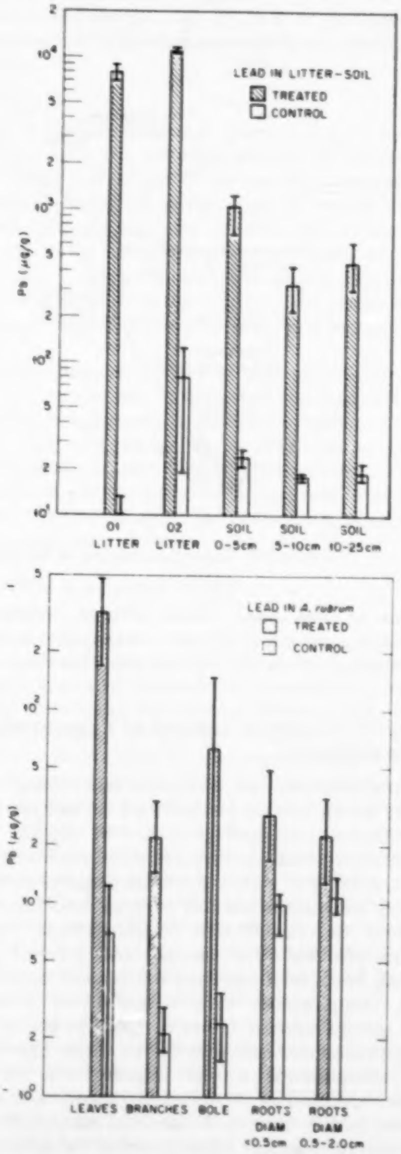

Figure 106. The distribution of lead in forest microcosm components at the conclusion of a 20-month experiment (Jackson et al. submitted).

Table 33—Indices of Microbial Activity Measured at Termination of a Microcosm Experiment Using Complex Heavy Metal Emissions from a Lead Smelter (Ausmus et al., Submitted).

Microbial parameter	Treatment	Soil 0-5 cm	Soil 5-10 cm
ATP concentration (ppm)	Contaminated	13.7[a]	4.2[b]
	Control	5.6	2.1
Bacterial density (10^8/g)	Contaminated	21.8[a]	10.2[a]
	Control	8.0	5.1
Fungal lengths (m/g soil)	Contaminated	75[a]	135[a]
	Control	1300	750

[a] Significantly different from controls at $P \leq 0.01$.
[b] Significantly different from controls at $P \leq 0.05$.

Table 34—Comparison of Three Parameters Measured in Control Replicates of Three Soil Core Microcosm Experiments.

Experiment	Parameters: CO_2 Efflux (µg/day)	Concentrations in Soil Water: NO_3-N (µg/1)	Concentrations in Soil Water: Ca (µg/ml)
1[a]	5.73 ± 0.19	32.4 ± 4.8	20.3 ± 1.7
2[b]	4.21 ± 0.35	30.1 ± 8.2	15.5 ± 5.6
3[c]	3.17 ± 0.99	27.3 ± 9.2	18.6 ± 6.1

[a] Jackson et al., in press.
[b] Ausmus and O'Neill submitted: O'Neill et al., submitted.
[c] Ausmus, Dodson and Van Voris, unpublished data.

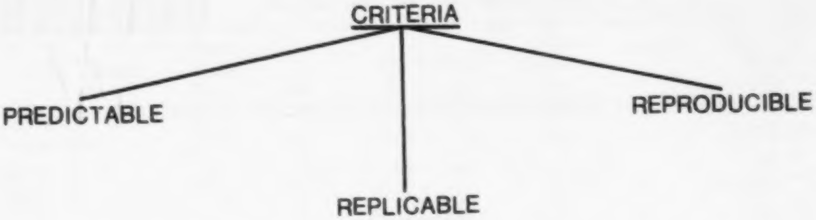

Figure 107. Criteria for microcosm quality control establishment.

period. Usually, this requirement necessitates equilibration of the experimental units prior to treatment with test substance (Figure 108). Second, confidence limits must be established for the measured parameters. Unique microcosms for each substance, or each dose rate are inapplicable. Third, reproducibility of parameters between control microcosms between comparable experiments must be reasonable.

Comparison of Reproducibility

Several comparable experiments have been conducted using the intact soil profile with and without aboveground vegetation. These experiments allow us to estimate reproducibility between experiments. An example of the findings is included as Table 34. We believe these microcosm units yield reproducible results as evidenced by the overlapping error terms for each of the three parameters among experiments.

Comparison of Replicability

Replicability among experimental units within a treatment class has been calculated during experiments using intact, homogenized, and sand-soil substrates. We have found that replicability is dependent upon substrate type and upon the parameter estimated. For example, CO_2 efflux was replicable in all substrates, but DOC efflux varied greatly among homogenized and sand-soil replicates at each sampling date and through time (Figure 109). Similar results were obtained for nine nutrients (Table 33). Results from grass microcosms established using these substrates were similar. We conclude that replicability of intact soil microcosms is greater than other substrates.

ACCURACY

Criteria

The criteria for determining microcosm result accuracy are dual (Figure 110). First, parameters indicative of ecosystem processes should be similar to those measured within comparable strata of the intact ecosystem. Second, the distribution and the transport rates of the test substance should mimic those which would occur in the source ecosystem. These criteria are the most difficult of those needed to establish the utility of microcosms in screening potentially hazardous substance. Only limited data have been collected to address this issue. The comparisons below represent our findings to date.

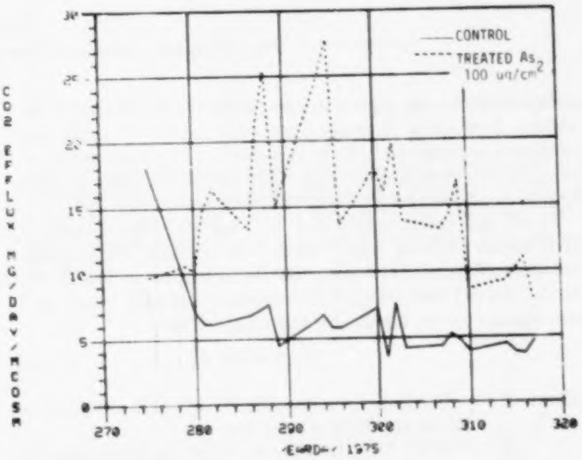

Figure 108. Equilibration of CO_2 efflux from pasture soil microcosms (Jackson et al., in press).

Comparison of Accuracy

Export of essential elements in water leached from intact, homogenized and sand-soil substrate microcosm were

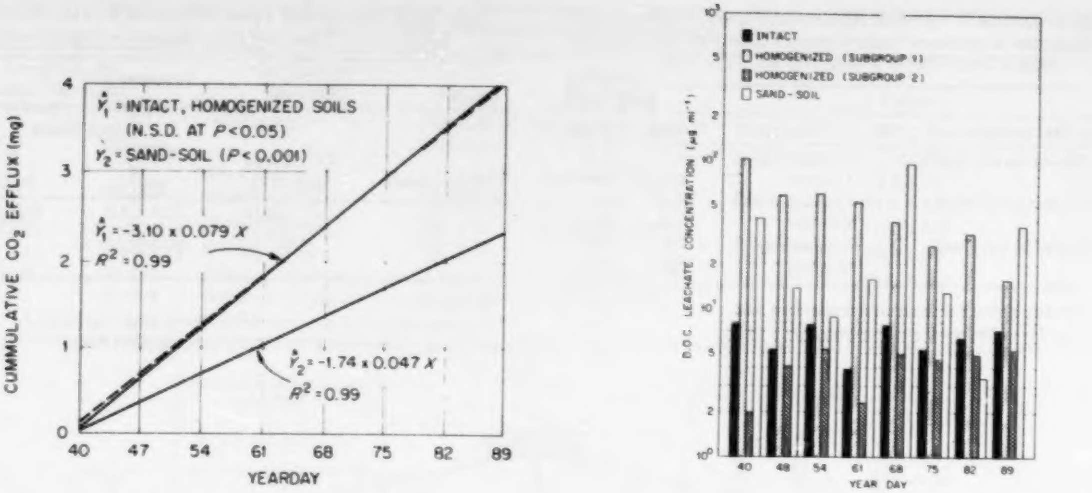

Figure 109. Replicability of two parameters monitored in microcosms. A CO_2 efflux; B. DOC efflux (Ausmus and O'Neill, submitted).

CRITERIA
SYSTEM SIMILAR
RESPONSE MIMIC

Figure 110. The criteria for determining accuracy of microcosm results

extrapolated to one year and compared. Table 35 shows the relative loss from homogenized and sand-soil substrates compared to intact soil. If we assume that the standard is intact soil, the comparability of other substrate types is poor. No ion is similarly lost from the three substrate types.

Intact coil microcosms can be quantitatively related to ecosystems. Using data from the source ecosystem, transport of nutrients below the rooting zone of the forest (60 cm depth) was related to transport through intact soil microcosms (5 cm depth) by the expressions:

$$Y_1 = 0.184\,X_1 \qquad (1)$$

where Y_1 = DOC, NH_3-N, NO_3-N, PO_4-P, or K transport to 60 cm depth (g/m²/yr), and
X = DOC, NH_3-N, NO_3-N, PO_4-P, or K transport to 5 cm depth in intact soil microcosms extrapolated to g/m²/yr.

$$Y_2 = 0.680\,X_2 \qquad (2)$$

where Y_2 = Ca, Na transport to 60 cm depth (g/m²/yr), and
X_2 = Ca, Na transport to 5 cm depth in intact soil microcosms extrapolated to g/m²/yr.

While these relationships alone are not conclusive proof of the accuracy of intact soil microcosm results, they are strong evidence that such is the case (Ausmus and O'Neill, submitted; O'Neill et al., submitted).

More conclusive evidence was obtained from forest microcosm experiments (Jackson et al., submitted). Using the data shown in Figure 106 and comparable data for cadmium, zinc, and copper, microcosm data were compared to the forest ecosystem under stress from the heavy metal deposition (see Watson et al., 1976; Jackson and Watson in press). Enrichment ratios, ER, were computed by dividing the heavy metal concentration of the treated microcosm components by the same metal concentration of the control microcosm component. Greatest enrichment of metals occurred in litter strata. Cadmium had greater ER for soil than did other metals, although Pb was greatly enriched. Values compared to those near the heavy metal source, were similar to the sampled ecosystem components (Jackson et al., submitted).

The most conclusive proof of accuracy to date has been the nonsignificant difference in field and microcosm data taken on similar dates.

PROBLEMS

Several problems remain in the evaluation of the utility of microcosms as screening tools to assess transport, fate and effects of potentially hazardous substances. Four problems seem important to the authors. These are: (1) the rate of ecosystem degradation cannot be calculated from loss rates of nutrient elements as yet, although in theory it is possible to do so (O'Neill et al., in press); (2) the microcosms have not been tested using organic effluents, singly or in combination; (3) absolute accuracy of microcosm results has not been proven; and (4) reproducibility among microcosm studies conducted using intact profiles excised during different seasons has not been established.

Some information is needed to conduct adequate screening of potentially hazardous substances. The mode of entry of the substance into the system is needed. The direct toxicity of the substance to research workers is needed in order

Table 35—**Comparison of Extrapolated Annual Export Among Substrates (O'Neill et al., Submitted).**

Substrate	Percent Extrapolated Intact Soil Microcosm Transport[a] Ca	K	DOC	PO_4-P	NH_3-N	NO_3-N	Mg	Na
Homogenized								
Block 1	108	74*	76*	100	35**	180*	100	128
Block 2	168*	76*	366**	400**	43**	30**	138*	450**
Sand-Soil	130*	20**	266**	20**	35**	18**	138*	200*

[a] Transport extrapolated from intact soil (g/m²/yr); Ca 5.8, K 5.4, DOC 6.2, PO_4-P 0.03, NH_3-N 0.66, NO_3-N 1.4, Mg 0.9, and Na 0.6.

*Significantly different from intact soil $P \leq 0.05$.

**Significantly different from intact soil $P \lesseqgtr 0.01$.

Table 36—CO_2 Efflux and CA Concentration of Soil Water on Comparable Dates in Forest Microcosms and the Source Forest Ecosystem (Ausmus and Ross, Unpublished Data)

Experimental Units	Julian Calendar Date	N[a]	CO_2 Efflux (mg/day)	Julian Calendar Date	N	Ca in Soil Water μg/ml
Forest	153	4	0.364 ± 0.063	184	4	10.67 ± 4.91
Microcosms	156	63	0.426 ± 0.014	188	9	8.78 ± 2.78
Forest	159	4	0.458 ± 0.063	190	1	6.56
Microcosms	163	63	0.462 ± 0.011	190	5	15.92 ± 7.54
Forest	167	4	0.491 ± 0.053	197	3	5.97 ± 1.11
Microcosms	170	63	0.534 ± 0.010	198	7	5.60 ± 0.95
Forest	174	4	0.669 ± 0.118	203	4	7.52 ± 2.00
Microcosms	177	63	0.573 ± 0.009	205	6	5.06 ± 0.96
Forest	188	4	0.477 ± 0.024			
Microcosms	191	63	0.556 ± 0.018			

[a] Number of replicate observations.

to practice safe laboratory experiments. The potentially targeted ecosystems should be identified if possible and used as sources of microcosm units. Lacking this information, forest or pasture systems should be used since more than 80% of the non-urban U.S. is one of these ecosystems.

High mobile invertebrates, macroinvertebrates, and vertebrates are not well represented within these small microcosm units. Studies directed toward the potential effects of these substances on these, due to their economic importance, or trophic position, should be conducted as biomagnification experiments, such as those described by Gillett (this volume).

RECOMMENDATIONS

Based on the several experiments conducted to date, we recommend the use of intact soil microcosms excised from targeted ecosystems and encased with or without aboveground vegetation, depending upon experimental purpose. We cannot recommend the use of artificial substrates, or homogenized soil reinoculated with biota for several reasons, including: (1) instability of these substrates with respect to parameters monitored; (2) limited replicability and accuracy of these units; and (3) proportionally higher cost in experimental set-up.

There is no substitute for choice of the appropriate experimental design for the experiment to be performed. We have found that a randomized incomplete block design with imcomplete factorial treatment arrangement is cost-effective and information rich. Analysis of parameter behavior through time under control and stressed conditions may furnish additional insights into the mode of effects on ecosystem processes and on response and recovery times which can be expected to levels of the stressor (Van Voris et al., 1977).

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Conceptual, Theoretical, and Empirical Considerations in Microcosm Design and Applications

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ABSTRACT

Conceptual framework, theoretical, and empirical considerations relevant to the design and application of studies of laboratory multispecies systems intended to evaluate the behavior and effects of toxic substances are presented. A conceptual framework is taken to be an outermost description of some domain of science, together with rules for its use. Explicit conceptual frameworks can be of value in problem perception, identification of approaches to problems, and evaluation of proposed solutions. A tentative, minimal conceptual framework for biology is briefly discussed, and its implications for design and conduct of microcosm studies in environmental toxicology are considered. A theoretical example of the effect of a toxicant on a simple multispecies system is presented to explicate particular conceptual framework considerations.

INTRODUCTION

With increased awareness of problems of toxic substances in natural ecosystems there has emerged in environmental toxicology much interest in studying the behavior and effects of toxicants in multispecies systems in the laboratory. The objectives and results of studies of toxicants in laboratory "ecosystems" or "microcosms" are intended to be relevant to the behavior and effects of toxicants in natural ecosystems. This raises the question of how such relevance is to be established. Natural biological communities, which together with the physical conditions of their existence form ecosystems, may be inconceivably complex. Laboratory models of ecosystems are not and cannot be designed to reproduce this natural complexity, this being neither the objective nor the method of modeling efforts in science. Rather, laboratory and other models are intended to abstract from nature major determinants of phenomena in which we are interested, so that we can achieve at least some initial understanding. But the ways in which we abstract from nature in designing multispecies systems in large part determine the results we will obtain and their possible relevance to problems in environmental toxicology. In this paper, we will only very generally note certain conceptual, theoretical, and empirical considerations we believe to be of some importance in the design and conduct of studies of laboratory multispecies systems.

CONCEPTUAL FRAMEWORK CONSIDERATIONS

We are all more or less aware that the ways in which we are innately and educationally prepared to view the world about us in large part predetermine how we observe natural phenomena, what phenomena we observe, and the meanings we attribute to observational experience. Philosophers of science have devoted much attention to the important roles of prior conceptual constituents and related social phenomena in the scientific process. Kuhn (1962), in "The Structure of Scientific Revolutions," familiarized scientists with ways in which scientific "paradigms" may determine problem perception, the legitimacy of approaches, and the acceptability of solutions. From somewhat different perspectives, Hanson (1958), Toulmin (1972), and others have also dealt with the conditions of scientific understanding and change.

Because they cannot be made explicit, social and scientific paradigms and psychological predispositions of individuals forming a scientific community are difficult to deal with rationally, no matter how much they may determine our scientific activities. We cannot specify all the social, scientific, and psychological determinants of the work and judgments of ourselves and other scientists. Even without this, scientists can abide with universal theories, if there are such for their domains of investigation. Without universal theory, there still remains some rather common, though often unarticulated, fundamental beliefs giving a certain unity to an area of science. Articulation of such beliefs in an explicit and general form could facilitate work, communication, and understanding among scientists. For, even when general statements of these beliefs are not accepted by all, the basis of arguments deriving from them can be seen and understood.

Gutting (1973) suggested a "conceptual framework" to be a set of descriptive statements and procedural rules subsuming the theories, models, and observational experience of some domain of science. Such a framework could hardly specify all that enters into the ways individual scientists proceed. But it could be of value in problem perception, identification of approaches, evaluation of preferred solutions, and rational communication of the bases for these. Warren, Allen, and Haefner (MS) tentatively proposed a minimal conceptual framework for biology, which consists of a set of four biological system generalizations and rules for their use. Abstract concepts in the generalizations allow them to be interpreted for different levels of biological organization. Warren and Liss (1977) employed such conceptual framework considerations in evaluating laboratory studies of individual organisms, populations, and multispecies systems in environmental toxicology. Here we will briefly discuss this framework and its implications for design and applicability to nature of multispecies laboratory system studies intended to evaluate the behavior and effects of toxic substances.

Within the context of this conceptual framework, a biological system at any level of organization is taken to be

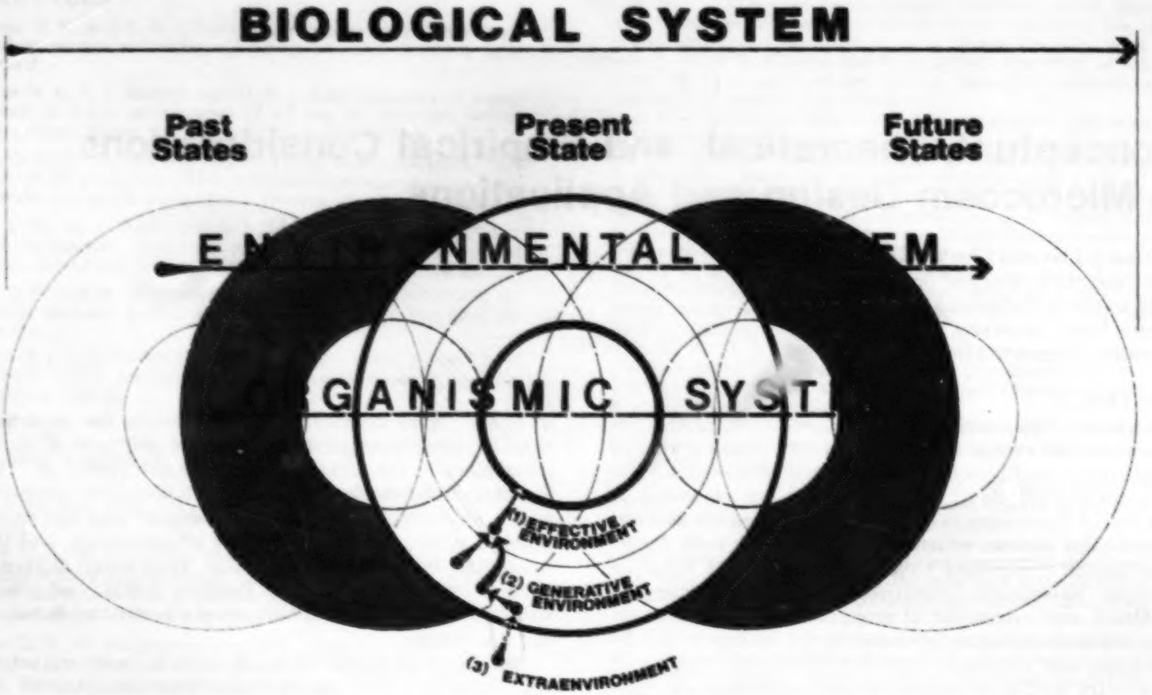

Figure 111. A biological system viewed throughout its existence as being composed of an organismic system together with its spatially and temporally co-extensive environmental system. After Warren, Allen, and Haefner (MS).

composed of an organismic system together with its environmental system co-extending in space and time. An organismic system is not what we perceive in any momentary space-time section, but rather that which persists from its origin through some present state to some future end (Figure 111). Thus, we view the individual organism as a system to be the entire life history trajectory, the developmental continuum of states from the zygote through reproductive maturity to death. Similarly, the biological community can be viewed as the entire series of successional states extending from a "pioneer" seral stage to some sort of "climax" stage. It is our conviction that a present state of any biological system can be fully understood only in the context of its past and possible future states.

A performance of an organismic system—something the system as a whole does—is determined by the capacity of the system and the immediately effective environment. Capacity is an abstract concept entailing all possible performances of an organismic system in all possible environments. We cannot conceive of a system exhibiting a performance for which it does not have the capacity. But we cannot determine, either theoretically or empirically, all possible performances of an organismic system in all possible environments. Yet, any possible performance of an organismic system can be abstractly accounted for as resulting from the capacity and the environment of the system. The concept of capacity encompasses the domain of possible kinds and levels of performances of an organismic system, thus helping to make clear what needs to be explained and the limited explanatory value of any particular measurement of system performance.

Structure, persistence, development, and replication are very general classes of organismic system performances that can be interpreted differently for different levels of biological organization. Growth is a performance of individual organisms, and production—or total tissue elaboration—can be considered a population level performance. Transport, accumulation, and degradation of toxic substances in multispecies systems are determined, in part, by the physical-chemical nature of the toxicants, but they are also performances of the organismic systems in which they are measured.

The capacity of an organismic system changes through time. Individual organism capacity changes as a result of development. The capacities of biological populations and communities change with evolution as well as with development. From some original potential capacity and depending upon the developmental or evolutionary environment, an organismic system comes to possess a particular sequence of realized capacities (Figure 112). The sequence of realized capacities of the system would differ in different developmental environments. For a particular capacity realized at any time, the kinds and levels of system performance that are exhibited will be determined by the then prevailing state of the environmental system. Any effective change in the realized capacity or in the environment must lead to some change in kinds or levels of possible performances.

An organismic system is composed of subsystems and it incorporates not only its subsystems but to some extent also their particular, level-specific environments (Figure 113). Furthermore, organismic systems or subsystems on each level of organization have their own level-specific capacities and performances.

Community structure, a performance of multispecies laboratory systems, can be viewed as the kinds and abundances of interacting species populations existing under a particular set of environmental conditions. This structure of

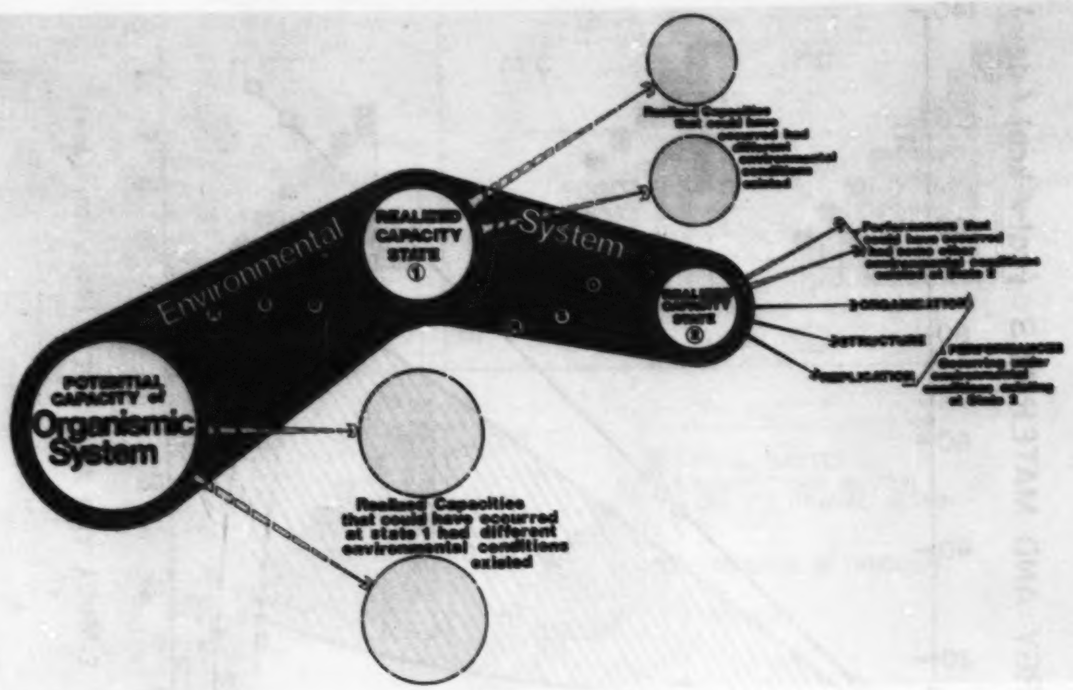
Environmental
System
REALIZED CAPACITY STATE ①
REALIZED CAPACITY STATE ②
POTENTIAL CAPACITY of Organismic System
Realized Capacities that could have occurred had different environmental conditions existed
Performances that could have occurred had some other environmental conditions existed at State 2
ORGANIZATION
STRUCTURE
REPLICATION
PERFORMANCES Occurring under environmental conditions existing at State 2
Realized Capacities that could have occurred at state 1 had different environmental conditions existed

Figure 112. A diagrammatic view of an organismic system showing that from some potential capacity the organismic system passes through a particular sequence of realized capacities jointly determined by its potential capacity and the actually prevailing environmental system. After Warren, Allen, and Haefner (MS).

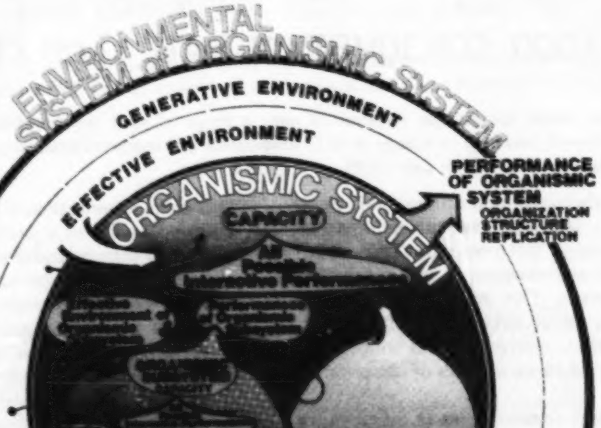
ENVIRONMENTAL SYSTEM of ORGANISMIC SYSTEM
GENERATIVE ENVIRONMENT
EFFECTIVE ENVIRONMENT
ORGANISMIC SYSTEM
CAPACITY
All Possible Interactive Performances
PERFORMANCE OF ORGANISMIC SYSTEM
ORGANIZATION
STRUCTURE
REPLICATION

Figure 113. Organismic subsystems and their level-specific environments, all of which are incorporated by an organismic system having its own particular environment. After Warren, Allen, and Haefner (MS).

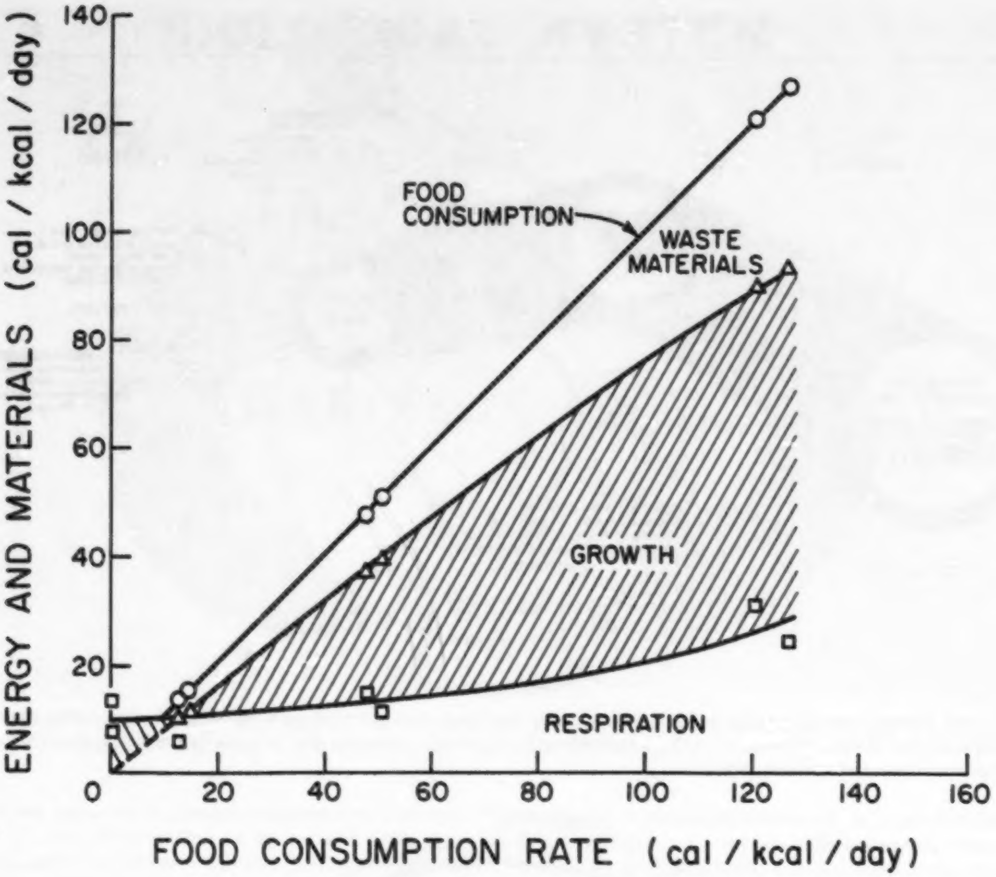

Figure 114. Scope for growth, determined over a range of food consumption rates, of juvenile largemouth bass held in aquaria at 20 C. Respiration and waste materials represent other fates of consumer energy. After Lee (1969).

a multispecies system is maintained operationally through the interactive performances of lower level subsystems—in this case, individual populations. In turn, population structure can be viewed as the abundances of individuals of different ages, sizes, and sexes. The interactive performances of these even lower level subsystems determine, under the prevailing set of their environmental conditions, the structure and other performances of each of the populations.

The organization of natural communities is much more complex than that of multispecies laboratory systems. A natural community can perhaps be thought of as being composed of subcommunities, such as a tree subcommunity, a shrub subcommunity, a grass-herb subcommunity, and a soil subcommunity. Each of these, in turn, encompasses trophic subsystems composed of closely interacting populations. At best, in terms of the number and kinds of populations as well as their interactions and functions, laboratory microcosms can represent only very low levels of organization of natural communities.

Capacity of an organismic system is a theoretical concept entailing all possible performances of the system in all possible environments. Any organismic system has the capacity for an indefinite number of kinds and levels of performances, and thus empirically, or even theoretically, the capacity of a system cannot be fully and directly evaluated. But either theoretically or empirically, a partial evaluation of the capacity of a particular system to perform—its scope for performance under specified sets of environmental conditions—can often be accomplished. Such a partial evaluation of capacity involves determination of the different levels of particular performances of an organismic system over ranges of values of selected environmental factors. Theoretical evaluations of scope for performance with models and higher level conceptual structures ought to go beyond empirical evaluations in explicating what the domain of performance is like and how toxic substances alter this domain.

Empirical determination of scope for performance can be illustrated with examples of the growth performance of individual organisms. Evaluations of scope for performance of individual organisms can be accomplished more readily than for population and multispecies systems. Growth of an organism is very much dependent upon the kind and quantity of food the organism consumes and the level of environmental factors under which growth occurs (Warren and Davis, 1967; Warren, 1971). The scope for growth of juvenile largemouth bass determined over a range of food consumption rates is shown in Figure 114. Had the temperature level, or any other important environmental factor, been different from that under which this scope was determined, the scope would have been different, as is shown for

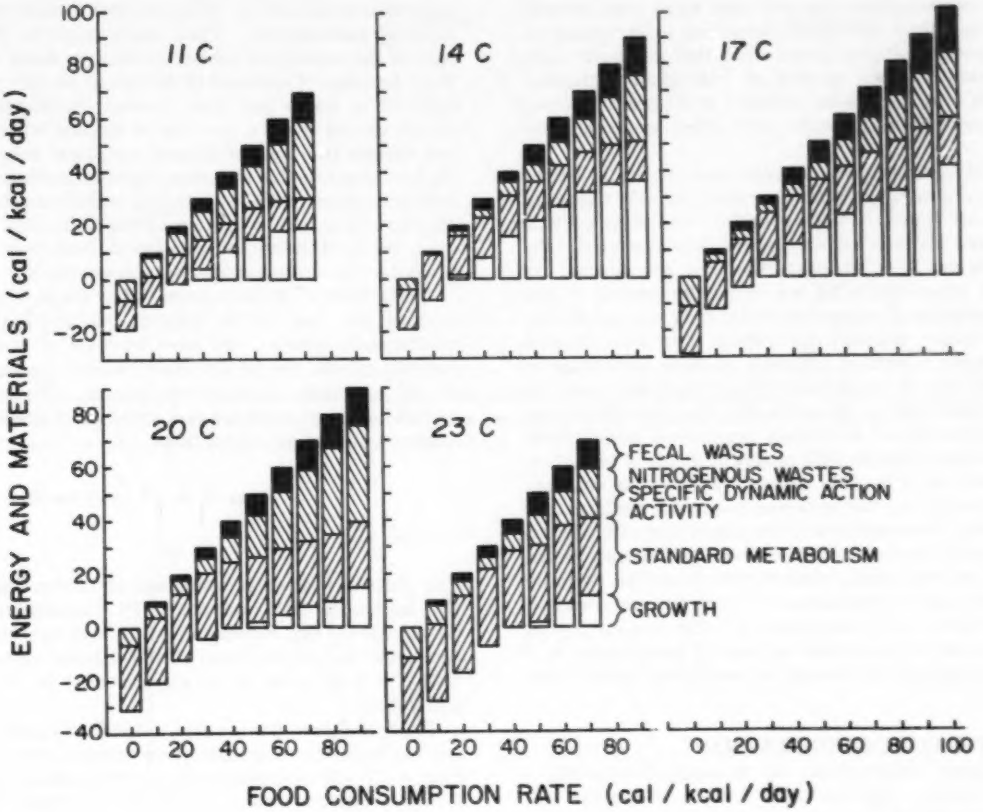

Figure 115. Relationship between scope for growth (open rectangles) and food consumption rate of juvenile coho salmon at different temperatures. Fecal wastes, nitrogenous wastes, specific dynamic action, activity, and standard metabolism represent other fates of consumer energy. After Averett (1969).

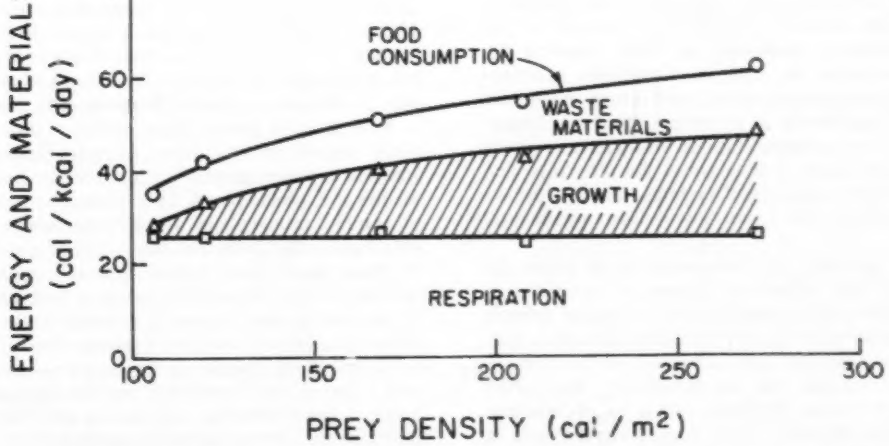

Figure 116. Scope for growth and food consumption of juvenile largemouth bass determined over a range of densitites of prey (mosquitofish) in experimental ponds. After Lee (1969).

juvenile salmon in Figure 115. The capacity for growth of either of these species entails all possible scopes for growth.

Under a particular set of environmental conditions, the food consumption of an organism and thus its growth will be determined to a large extent by the density and availability of its prey species (Figure 116; see also Holling, 1959; Ivlev, 1961). Rate of accumulation and effects of toxicants obtained through the food will also be dependent upon food consumption rate and thus upon prey density. Furthermore, a toxic substance, according to its concentration and mode of action, could alter the metabolic state, food consumption, and growth of individual organisms. Such effects are likely to be different at different levels of food availability, temperature, and other environmental factors.

In natural systems, prey densities and availabilities and other environmental conditions are continuously changing. Organisms are generally living under conditions of food limitation and levels of environmental factors such as temperature, oxygen, and moisture that are not optimal for growth and reproduction. If we know the growth or any other performance of organisms under only one set of conditions, we cannot say very much about their performances and the possible effects of toxicants on these performances under other sets of conditions. Thus, there is a need to evaluate, empirically or theoretically, the persistence and other performances of individual organisms, populations, and multispecies systems over ranges of levels of important environmental factors. Our general interests in environmental toxicology are not with any particular performance but rather with the extent that toxic substances alter the domain of possible system performances. This domain is the capacity of an organismic system, which can be partially evaluated through determination of scope for performance. The importance of evaluation of the capacities of organismic systems is evident so long as one accepts as *a priori* the conceptual framework we have considered in this section.

THEORETICAL CONSIDERATIONS

A universal description of biological systems—organismic systems together with their level-specific, coextensive environmental systems—should in some very general and abstract way incorporate all that is most essential to at least initial explanation and understanding of the nature of biological systems. The conceptual framework we have considered is a tentative attempt to provide such a description. As such, it should make clear the general relationships among environments, capacities, and performances of organismic systems. In so doing, it should further make clear explanatory problems we face, possible approaches to these problems, and what adequate solutions might be like. But the generality and abstractness necessary for a conceptual framework to encompass and say important things about any biological system whatsoever make it unsuited to say particular things about the structure and behavior of particular organismic systems. To do this, we need theories, models, and other lower level conceptual constructs.

We need, for example, to know something about the possible behaviors and effects of classes of toxicants in multispecies systems of different kinds. Adequate models and conceptual constructs should not only elucidate particular dynamic and steady-state behaviors of the populations in such systems but also the domains of population and system behavior under different sets of environmental conditions (see also Holling, 1973). And it should help to make clear what effects toxicants may have on such domains of behavior as well as on particular behaviors. Inductively from the results of experiments on the behavior and effects of toxicants in multispecies laboratory systems, we can only hope to obtain empirical generalizations. But, according to the composition and environmental conditions of these multispecies systems—according to the design of the microcosm studies—resulting data and empirical generalizations will be different and sometimes even apparently contradictory. They apply more or less directly only to the conditions under which they were determined, their domains of application are apt to be limited, and it is difficult to know just how limited. Adequate biological theory should make it possible to deduce sets of theorems translatable into sets of diverse empirical generalizations, thus helping to make clear the interrelationships among the generalizations and the boundary conditions for their application to nature where problems with toxic substances exist. We need theory for such logical deductive unification as well as for more particular explanations.

The effects of differences in the levels of environmental factors on the performances of populations and multispecies systems, and how these differences influence toxicant effects, can be illustrated with a theoretical example of a simple multispecies system. The multispecies system can be represented as a sequence of predator-prey or resource-utilization interactions:

$$I \rightarrow R \rightleftharpoons P \rightleftharpoons H \rightleftharpoons \underset{\overset{\uparrow}{T}}{C} \rightleftharpoons E$$

The defined system is composed of interacting carnivore (C), herbivore (H), and plant (P) populations and light resource level (R). Fishing effort (E) and light input rate (I) constitute the environment of the defined system. A toxicant T is here taken to act directly only on the carnivore population.

Phase planes and interrelated isocline systems representing this sequence of predator-prey interactions are shown in Figure 117. On each phase plane, prey biomass (or resource level) is plotted on the x-axis and predator biomass (or utilizer level) on the y-axis. The descending curves parameterized by light input rate (low I, med I, high I) on each phase plane are the prey or resource isoclines. Every prey isocline on each phase plane is a set of biomasses (or levels) of predator and prey at which the rate of change of prey biomass with time is zero ($dR/dt = 0$ on the P-R phase plane, $dP/dt = 0$ on the H-P phase plane, $dH/dt = 0$ on the C-H phase plane). The ascending curves on each phase plane are predator or utilizer isoclines. Each predator isocline is a set of biomass (or levels) of predator and prey at which the rate of change of predator biomass with time is zero ($dP/dt = 0$ on the P-R phase plane, $dH/dt = 0$ on the H-P phase plane, and $dC/dt = 0$ on the C-H phase plane). Each predator isocline is parameterized by a particular herbivore biomass on the P-R phase plane, by a particular carnivore biomass on the H-P phase plane, and by a particular level of fishing effort and a particular concentration of toxicant T on the C-H phase plane. Each intersection of a predator and a prey isocline, where the rate of change of both predator and prey biomass with time is zero, is a steady-state point. With the phase planes and isocline systems shown in Figure 117, some effects of changes in the levels of environmental factors I and E and toxicant T on the biomasses and persistence of the interacting populations and the structure of the multispecies system can be demonstrated.

The interrelated isocline systems and the trajectories on the phase planes were constructed from sets of graphs or response functions that represent performances of each of the interacting populations. Booty and Warren (MS) provide a detailed description of the graphical derivation of predator and prey isoclines on all phase planes. Here only the derivation of the predator and prey isoclines on the C-H phase plane will be shown. The same method is used to determine

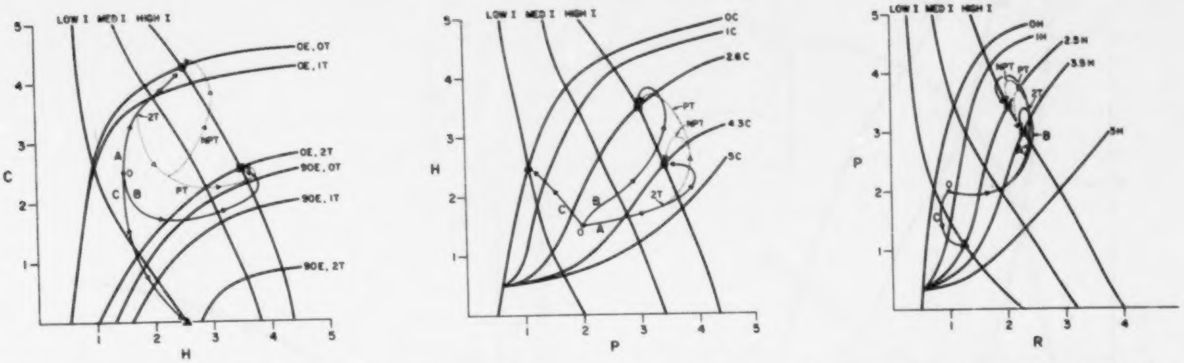

Figure 117. Phase planes and isocline systems used to represent steady-states and trajectories of biomasses of carnivore (C), herbivore (H), and plant (P) populations and light intensity (R) at different rates of light input (I) and different levels of fishing effort (E), when toxicant is absent (OT) and when it is present at concentrations of 1T and 2T.

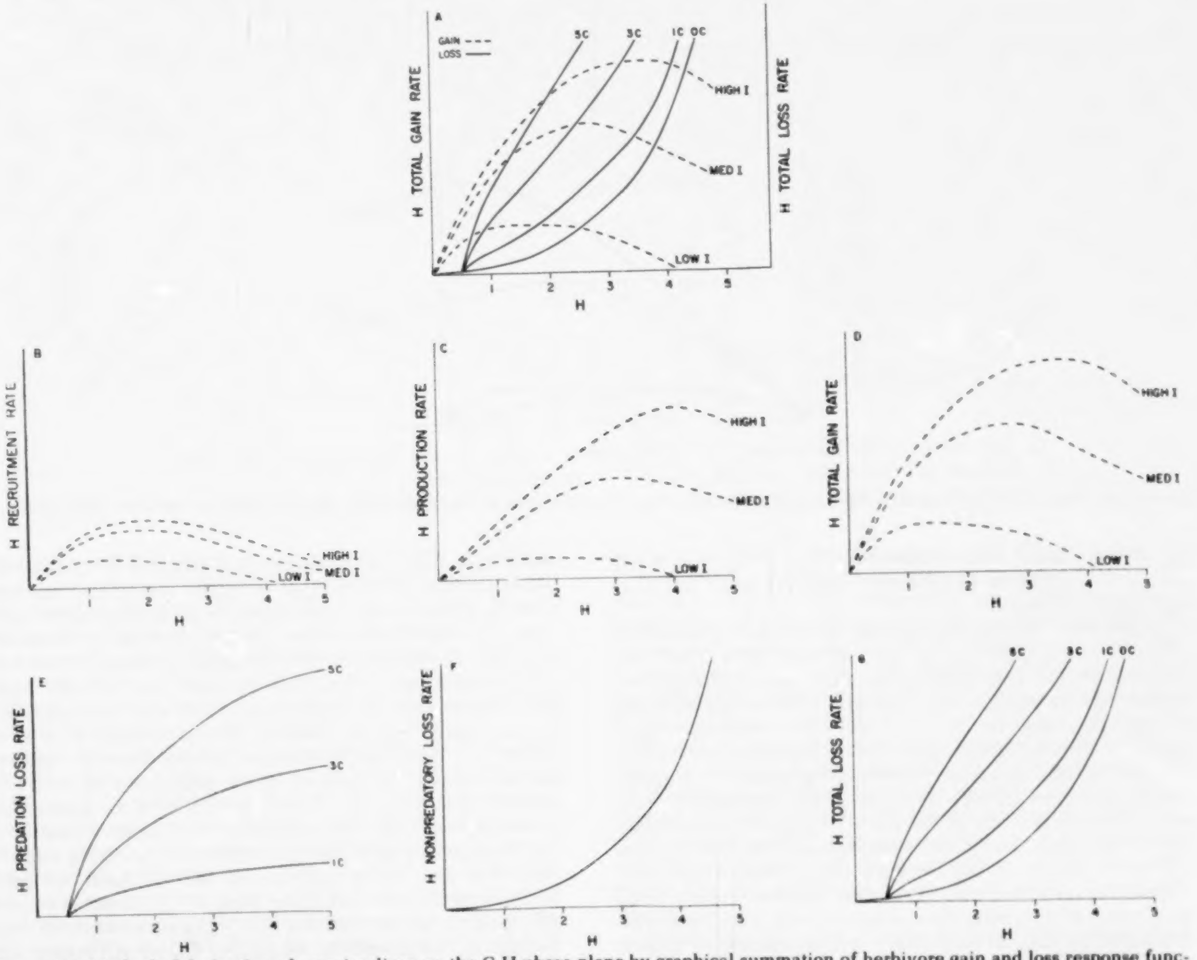

Figure 118. Method of derivation of prey isoclines on the C-H phase plane by graphical summation of herbivore gain and loss response functions.

the forms and positions of the isoclines on the P-R and H-P phase planes.

The position and form of the prey isoclines on the C-H phase plane are derived from response functions that represent performances of the herbivore population (Figure 118). The rate of change of herbivore biomass with time (dH/dt) is determined by the rate that the herbivore population gains biomass and the rate that it loses biomass. Rates of gain of herbivore biomass are represented by curves of recruitment and production. Consumption by carnivores

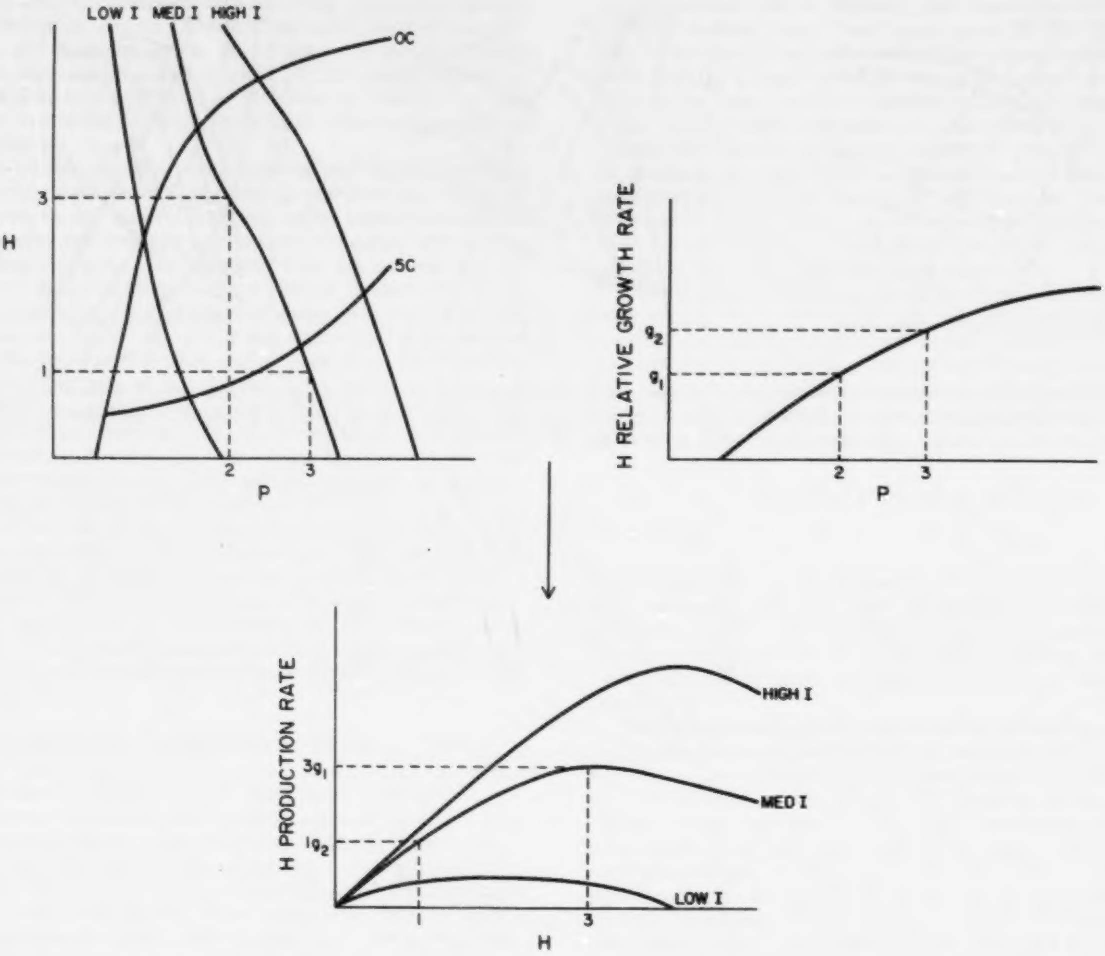

Figure 119. Method of derivation of the herbivore production gain curves used in constructing the prey isoclines on the C-H phase plane.

(predation losses) and nonpredatory losses such as those owing to emigration and disease represent rates of loss of herbivore biomass.

Herbivore recruitment (Figure 118B) and production (Figure 118C) gain curves are derived from the prey isoclines on the H-P phase plane and curves relating herbivore relative recruitment rate and relative growth rate to plant biomass. Derivation of herbivore production gain curves is illustrated in Figure 119. A herbivore production gain curve and a herbivore recruitment gain curve are determined from each prey isocline. Each production and recruitment gain curve is, therefore, parameterized by a particular light input rate. The recruitment and production gain curves of the herbivore for each rate of light input are summed to generate a set of curves representing the total gain rates of the herbivore (Figure 118D). Similarly, the nonpredatory loss curve (Figure 118F) is summed with each predation loss curve (Figure 118E) to generate a set of curves representing herbivore total loss rates (Figure 118G).

The prey isoclines on the C-H phase plane are defined as the sets of biomasses of carnivore and herbivore at which $dH/dt = 0$. When $dH/dt = 0$, the total rate of gain of herbivore biomass is equal to the total rate of loss of herbivore biomass. If curves representing total gain rates (Figure 118D) and curves representing total loss rates (Figure 118G) are plotted on the same graph (Figure 118A), the intersections of a gain curve identified by a particular rate of light input (I) with a series of loss curves identified by carnivore biomass (C) define a set of biomasses of carnivore and herbivore where $dH/dt = 0$. That is, each rate of light input generates and so identifies a prey isocline on the C-H phase plane (Figure 117). In general, this procedure is used to derive prey isoclines on all phase planes. Rates of light input are initially introduced as the gain terms of R and so become the identities of prey isoclines on all successive phase planes. This is a consequence of constructing prey isoclines on any particular phase plane by utilizing recruitment and production gain curves derived from the prey isoclines of the previous plane. Rate of light input is not the only possible parameterizing identity of prey isoclines. Any biological, physical, or chemical factor affecting the response functions of a population would become an additional parameterizing identity of the prey isoclines derived from the population's response functions.

Essentially the same procedure is used to construct predator isoclines on the C-H phase plane: graphical summation of carnivore gain curves (recruitment and production) and loss curves (yield and nonpredatory losses), as shown in

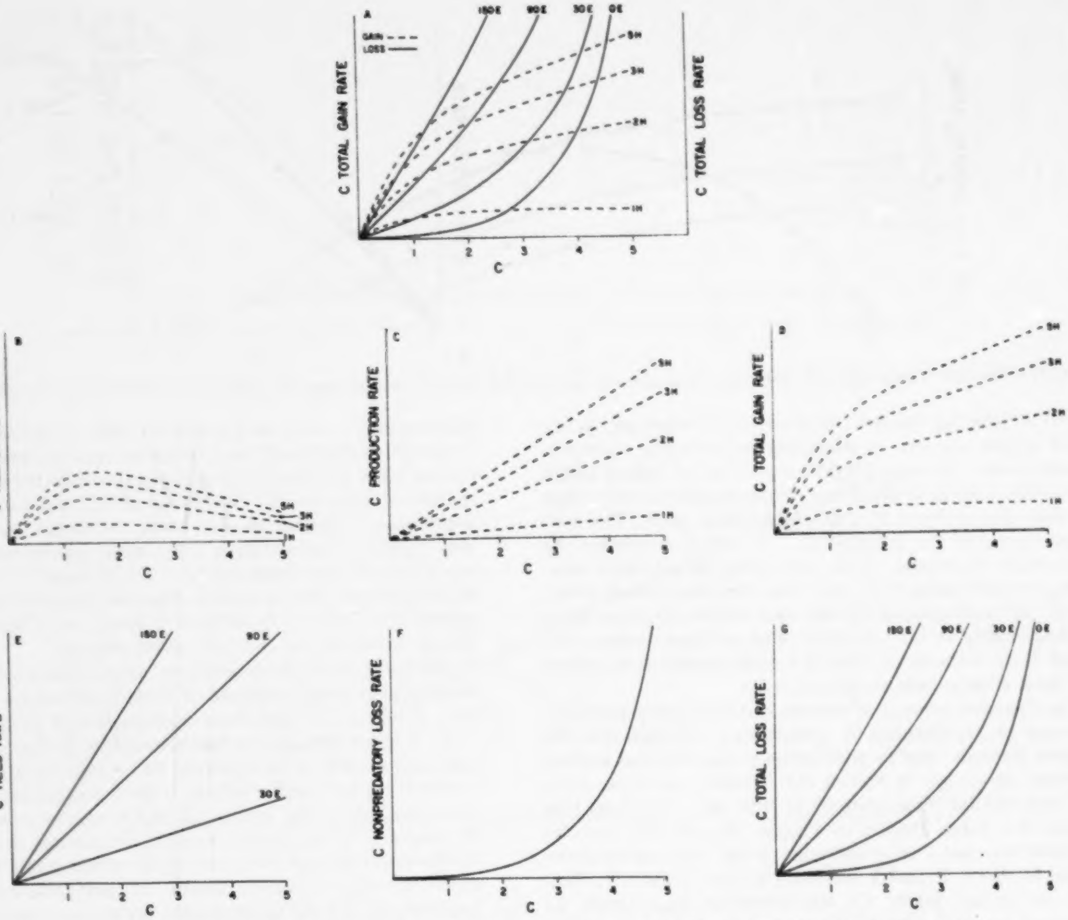

Figure 120. Method of derivation of predator isoclines on the C-H phase plane, for a toxicant concentration of OT, by graphical summation of carnivore gain and loss curves. The predator isoclines on the C-H phase plane are the sets of biomasses of carnivore and herbivore where dC/dt = 0.

Figure 120 for a toxicant concentration of OT. Carnivore production (Figure 120C) and recruitment (Figure 120B) response functions are solely derived, respectively, from a relationship between carnivore relative growth rate and herbivore biomass (Figure 121) and a relationship between carnivore relative recruitment rate and herbivore biomass. This method of determining carnivore gain response functions facilitates construction of predator isoclines.

Carnivore loss curves are represented by yield response functions, parameterized by level of fishing effort (Figure 120E), and nonpredatory losses (Figure 120F). Carnivore recruitment and production gain curves for each herbivore biomass are summed to generate a set of curves representing carnivore total gain rates (Figure 120D). Similarly, the nonpredatory loss curve is summed with each yield curve to generate a set of curves representing total loss rates of the carnivore (Figure 120G). If curves representing total rates of gain (Figure 120D) and curves representing total rates of loss (Figure 120G) are plotted on the same graph (Figure 120A), the intersections of a loss curve identified by a particular level of fishing effort (E) with a series of gain curves identified by herbivore biomasses (H) define a set of herbivore and carnivore biomasses where dC/dt = 0. Thus each level of fishing effort generates and, therefore, identifies a predator isocline on the C-H phase plane (Figure 117).

In Figure 122A, the relative growth rate response of the carnivore is shown to be reduced by increasing concentrations of T from 0T to 2T. The carnivore relative growth rate response is used to construct the carnivore production gain curves (Figure 121) and, consequently, toxicant concentration as well as herbivore biomass will parameterize the production response functions. In Figure 122B, a toxicant concentration of 2T is shown to be acutely toxic (toxicant induced mortality) to the carnivore. Toxicant induced mortality represents an additional loss function. Toxicant concentration parameterizes both the production and the toxicant induced mortality response functions and, therefore, becomes an identity of the carnivore total gain and loss curves and the predator isoclines on the C-H phase plane (Figure 117).

For a given rate of light input, level of fishing effort, and toxicant concentration, a single steady-state point exists on each phase plane, the set of these defining mutual steady-state biomasses of C, H, P, and R. In Figure 117, there are shown the steady-state points on each phase plane that define the mutual steady-state biomasses of C, H, P, and R—the steady-state structure of the defined system—at high I, OE, OT (circles), high I, OE, 2T (squares), and low I,

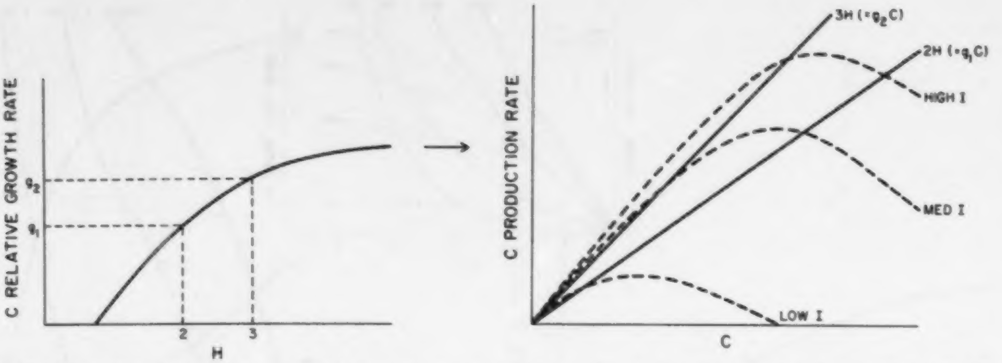

Figure 121. Method of derivation of the carnivore production gain curves used in constructing the predator isoclines on the C-H phase plane.

90E, 2T (triangles). Each of the three steady-state points in a set (of circles, squares, or triangles) generated by a particular combination of rate of light input, level of fishing effort, and toxicant concentration is a two-dimensional projection of a four-dimensional system steady-state point. The particular values of the parameters I, E, and T determine the steady-state biomasses of the interacting populations composing the defined system and, thus, the steady-state structure of the multispecies system as a whole. Because there are conceivably an infinite number of particular values of I, E, and T, an infinite number of system steady-state points and, thus, steady-state structures exist.

The dynamic aspects of system and subsystem performance can be represented by trajectories that describe the changes through time in population biomasses and system structure, as shown in Figure 117. These trajectories have been determined from systems of total gain and total loss curves like those shown in Figure 120, except that the effects of toxicant T were included. If the levels of environmental factors I, E, and T are fixed at high I, OE, OT, then from an initial point, O, simultaneous trajectories of biomasses on each phase plane (A trajectories) converge upon the points locating the steady-state biomasses of C, H, P, and R (solid circles). The A trajectory on each phase plane is a two-dimensional projection of a four-dimensional trajectory that converges upon the system steady-state point.

But if, after some period of time, a persistent toxicant (PT) directly affecting only carnivore C is introduced at a concentration of 2T, then the trajectories are perturbed and converge upon the points defining the steady-state biomasses of C, H, P, R at high I, OE, 2T (solid squares). These are the same steady-state points upon which the trajectories converge if the toxicant is present initially at a concentration of 2T (B trajectories). If a nonpersistent toxicant (NPT) directly affecting only carnivore C is introduced at a concentration of 2T at the point so marked on the A trajectories, these trajectories are perturbed and progress toward the steady-state points generated by high I, OE, 2T, initially by the paths of the trajectories resulting from introduction of the persistent toxicant. As the concentration of the nonpersistent toxicant in the system decreases, however, the trajectories begin to return to the steady-state points generated by high I, OE, OT on each phase plane. Through its effects on the carnivore population, toxicant T indirectly influences the performances of all populations and the structure of the defined system as a whole.

Now, if the levels of environmental factors I, E, and T were fixed at low I, 90E, 2T, trajectories originating from point 0 on each phase plane converge upon the steady-state biomasses of C, H, P, and R at low I, 90E, 2T (C trajectories). Under this set of conditions, the prey isocline parameterized by low I and the predator isocline parameterized by 90E, 2T do not intersect on the C-H phase plane, and the carnivore population is driven to extinction. Although carnivore C was capable of persisting at a toxicant concentration of 2T when I and E were fixed at high I, OE (B trajectory on the C-H phase plane), the carnivore does not have the capacity to persist at 2T when I is reduced to low I and E increased to 90E (C trajectory on the C-H phase plane).

We have, in a theoretical way, considered a very simple ecological system composed of three populations (P, H, C), light resource (R), and three environmental parameters (I, E, T). If other populations had been added to this system, its capacity as well as its dynamic and steady-state behaviors would have been different, even for the same levels of the same environmental factors. Or, if the populations and thus the capacity of the system remained the same, but the environmental factors were changed—either qualitatively or quantitatively—different levels of performance of the populations and the multispecies system as a whole would be manifested. The effects of toxicants on the performances of populations and multispecies systems will generally be determined by the kinds and levels of other environmental factors as well as by the nature of the organismic system of interest. The most general interest in laboratory microcosms must lie in knowledge of the domains of their dynamic and steady-state behaviors and how toxic substances alter these domains.

EMPIRICAL CONSIDERATIONS

Conceptual framework, theoretical, and empirical considerations in science do not deal with clearly distinct ways of understanding, even though maintaining some distinction helps to ensure adequate emphasis on each. Recent developments in the philosophy of science have shown the distinction between "theoretical languages" and "observational languages," emphasized by the logical positivists, to be quite uncertain. Our theoretical concepts are experience-laden and our empirical concepts are theory-laden. What we may take to be empirical considerations are deeply embedded in our total conceptual natures. Even apart from problem perception and the giving of meaning to the raw data of perceptual experience, relatively high level conceptual constructs provide underlying bases for when, where, how, and on what we make observations. Certainly this is evident in the very notion of "design" of experiments, with which we are here concerned. Moreover, however much we may formally recognize it, the application of laboratory results to the solution of problems in nature demands

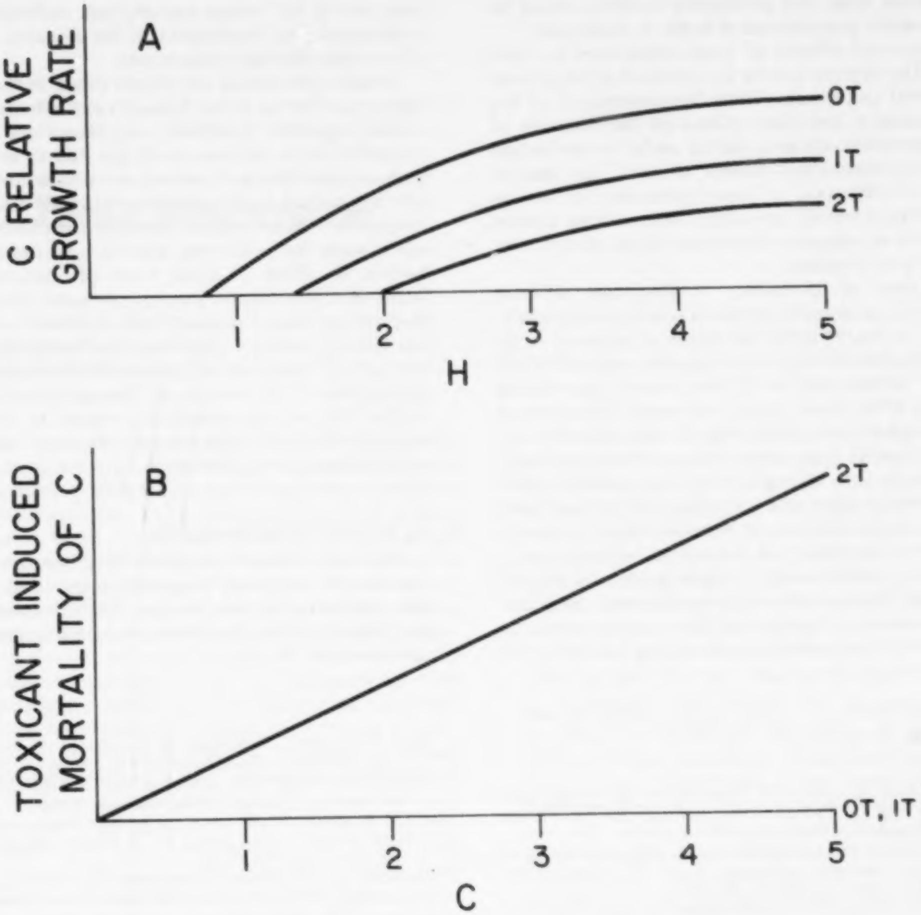

Figure 122. (A) Direct effects of toxicant T on the relationship between carnivore, C, relative growth rate and herbivore, H, biomass at concentrations of OT, 1T, and 2T. (B) Toxicant T is shown to have a direct effect on the mortality rate of the carnivore, C, at a concentration of 2T but not at 0T and 1T.

relatively high level conceptual constructs, about nature, about our experiments, and about the relationships between these. It is primarily for these reasons that we have first placed so much emphasis on conceptual framework and theoretical considerations.

In designing a laboratory multispecies system, we are directly involved not only in determining environmental conditions but also in determining the capacity of the multispecies system itself. We determine the capacity and thus the domain of possible performance of the multispecies system by bringing together populations or groups of organisms having their own capacities. Each differently designed laboratory multispecies system has a unique capacity. Its capacity will be different not only from other laboratory systems but also from natural systems. What is there, then, that we can say about the behavior and effects of toxic substances in nature on the basis of laboratory studies?

Multispecies laboratory systems will be most useful in environmental toxicology when we are able to design them to have capacities for general and important performances—such as structure and persistence—occurring in nature. Then we will have more reason to believe that the behavior and effects of toxic substances introduced into such systems will be indicative of what may occur in nature. We should select for inclusion in laboratory systems species that are representative of different classes of organisms (i.e., populations of plants, herbivores, carnivores, and decomposers), and interactions (i.e. predation, competition, commensalism, and mutualism). For persistent population systems and thus persistence of a designed multispecies system as a whole, suitable environmental conditions and resources must be provided for completion of the life histories, including reproduction, of individuals composing the populations, thus permitting the populations to develop age and size structure. Populations at each step in the food chain or web must have the capacity to provide sufficient energy and material resources to support other populations dependent upon them under the environmental conditions provided. Only with populations of small, rapidly reproducing organisms having relatively short lifespans is it usually practical to develop persistent multispecies systems in the laboratory. Rather large and productive systems would be necessary to sustain populations of birds or mammals.

The behavior and effects of toxic substances in such systems should be determined for a number of different sets of environmental conditions. Such demonstrations of the behavior of toxicants and their effects on the domains of behavior of organismic systems can be useful in evaluating the potential environmental hazard of toxic substances. Fry's (1947) classification of environmental factors as lethal, controlling, limiting, stressing, and masking factors should be useful in selecting important kinds of environmental factors to be studied.

Different kinds of laboratory multispecies systems should be studied, each under different sets of environmental conditions. In determining the kinds of systems to be studied, classification of organismic system properties and environmental factors will be of the utmost importance. From the results of all these studies, we must induce sets of empirical generalizations pertaining to the behavior and effects of a particular toxic substance or classes of toxic substances. The domain of application of a particular empirical generalization cannot be very great if it is based only on a particular study. But if it is based on many properly conceived studies, an empirical generalization can have a larger domain, one more nearly likely to pertain to nature. Even so, many different empirical generalizations, some apparently inconsistent, will probably be necessary to cover a wide range of empirical experience. It is then that we must seek theory for further explanation, understanding, logical unification, and clarification of the domains of applicability of our empirical generalizations.

Finally, we should not expect much in the way of quantitative prediction of the behavior and effects of toxicants in natural systems. Certainly our laboratory systems must generally leave out too much for this to be possible. The unique capacities and various environments of organismic systems lead to such vast arrays of kinds and levels of performances that we are not likely to soon have theoretical or other ways for achieving precise quantitative prediction. Rather, we think, it is the kinds of relationships and the forms of relationships among biological identities and the changes in these resulting from toxicants with which we can hope to deal by theoretical and empirical means. It is this sort of "qualitative," rather than strictly quantitative, application of the results of laboratory ecological system studies that we can reasonably expect to be successful in understanding the behavior and effects of toxic substances in complex natural systems.

ACKNOWLEDGEMENTS

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Round Table Discussion

Dr. Robert E. Van Hook, Chairman
Oak Ridge National Laboratory,
Environmental Sciences Division,
Oak Ridge, Tenn.

VAN HOOK: We have heard quite a range of things today on microcosm systems, looking on the one hand at fate, and on the other hand at effects. We have heard about practical and theoretical systems, about sizes ranging from a centimeter to meters in width and breadth, about natural systems, and constructed systems. We have also looked at complexity from a very few to quite a number of components, and each of these aspects can be argued, have been argued today and will be argued in the future. I don't think we will settle them in the next few days. There are three or four things that occurred to me that I would like to say. *First* (and I will get some debate), soil is the most important component of these terrestrial microcosms and it is the component we know least about in terms of putting it in a box, making some measurements and saying something about the validity of those measurements. *Second,* we appear to be in relatively poor standing with respect to effects as compared to transport. To say it another way, we know more about how to build a box to tell about transport, degradation and fate then we do about effects. The question that comes to my mind is, "What do these transport data tell us?" So we know it degrades in so many hours. So we know that such and such a percent goes to compartment X. I guess the question in mind is, "So what?" *Lastly,* microcosms should be designed with specific questions in mind. There will not be a universal microcosm and we very definitely need to know before we build the box what questions we are asking. We need to have some idea of the relation of the microcosm to the real world. If it has no relation, that may not be bad. We have microcosms for comparative purposes. We can look at the behavior of various chemicals under similar situations and say something about how they behave relative to some known. On the other hand, if they depict the real world, all the better, for we can then say something about the meaning of what we have observed, relative to the real world.

DR. FRIEDA TAUBA: You are correct in saying that our understanding of effects at the present moment is considerably worse than our understanding of transport. However, one thing that has been holding up the study of effects is the inadequacy of understanding the transfer mechanisms and particularly the problems of many of the toxicants or chemicals regarding binding on sediments or volatility. Many of the kinds of studies that need to be done on effects can now be started, whereas before there were losses of toxicants that were not being taken into account. Certainly the bound residue problem has to be dealt with if you are going to be able to say how much of the chemical is around in an effective form.

There are a couple of reasons why effects have not shown up in a lot of the studies to date. One brought out very nicely by Dr. Liss is that you cannot "unemploy" a system. If you have a system from which you are not harvesting anything or putting any pressure on the population whatsoever, the population will not be very responsive, won't be able to reach its growth or reproductive potential, and so the toxicant kills a few animals off. If you want to look at effects, you are going to have to wait enough time for at least growth to occur. If you want to look at population changes, you must wait until reproduction occurs.

DR. SYDNEY DRAGGAN: We have been used to looking at effects on individual organisms or populations and we must be more sensitive to demonstration of effects on ecosystems. One problem is how we perceive an ecosystem. If we start looking at an ecosystem as if it were an organism, we will have a better chance of demonstrating these (system) effects and convincing others that ecosystem effects can be demonstrated, that they are important, and that they have a bearing on what's going on.

DR. LARRY COLE: It might be important to define why we are here: Whether we are here to study toxic substances or things that might harm the environment, or if we are here just to study the environment as a whole, which is a different question. Let's assume that we are here to study toxic substances. Next we should define what type of toxicity. If you believe that we do not at the present time have means to study acute toxicity, that is quite incorrect. If you believe we do not have a method for studying chronic toxicity, that is wrong too. We can study chronic toxicity if we take a long period of time. If you believe that we do not have a method of studying chronic toxicity in a short period of time, then I think that is true and the main concern as far as the effect of toxic substances.

One other thing, regarding an environmental concept, is that the environment consists of both the abiotic and the living system. The toxics only affect the living part. It is hard to say that the soil is an organism. Although most of the toxicant is deposited on the soil. Are we going to study the effect on a non-living thing?

DR. JAMES GILLETT: Larry, this organismic concept is simply that you can look at your jug or our tank or Ralph's [Nash's] big box as a system which has certain properties that have the same characteristics as an organism. It has nitrogen transport and flow, as an organism has a nitrogen metabolism. We are not saying that they are the same, but they are adequate for discussion or analogy. We would like to think of a clinical analogy where you would devise tests of that system just as we would devise tests of an organism. It would be nice to have something like a glucose Clinatest® that would tell us when a system is "diabetic" or short of DOC the same as we have for a person. When we have a chemical which might stress that system, e.g., put that "person" in jeopardy, we would like to be able to assay the same for an ecosystem by methods that would be just as quick. We do not have anything like that yet, but we have talked about them in this context.

DR. BEVERLY AUSMUS: If I can take that heresay one step further, I met last week with a group of people from our medical department at Battelle. We were talking about the commonality between environmental toxicology (which I'm not sure [if] I know what [it] is yet), and health effects of classical toxicology. The health people have the same trouble; they have Ames tests which are supposed to indicate whether or not something might be toxic to human beings. However, there seems to be very little, if any, direct correlation between the Ames tests and actual effects, because we are not allowed to dose people. So we are looking for something that you could use as a diagnostician might if he were examining someone who was ill. One of the more intriguing questions we have come up with lately has been, "Can we be turned into internists for ecosystems?" I know when I am getting a sinus infection, not simply because my head eventually swells up but because my fever spikes two or three days before the swelling begins. If we can come up with the kinds of diagnostic tools, which is exactly what you meant by the glucose test, it seems to me that we could come up with at least a more reasonable understanding of what a system needs to do to persist. Unless a system persists, the animals in them do not, and we are one of those animals that I am a little concerned about it.

DR. COLE: Again, when you say the term "system," what are you talking about? A lot can exist and not necessarily affect me, but maybe it will. If the food that I need to eat does not exist, then of course that would hurt me quite badly. So when you talk about this part of the ecosystem being like an organism, I think you'd better define it. Do you have something more specific in mind, a parameter like calcium? What other parameters are you talking about? CO_2?

DR. GILLETT: Productivity. At the farm site here we are going to get so many hundred pounds of corn per acre. If SO_2 is excessive, we are not going to get 300 pounds per acre. We are going to get 240 or 110 or zip. If we are talking about forests, we have millions of acres of forest which, if they are impacted by hundred ppm ozone, will have zero productivity. Productivity is what we live by.

DR. COLE: Can you look at that in a short period of time? Can you look at the growth of a corn field in the time span that you need? I think we are going to have to define the time span and the space and what it is that we want to measure very quickly or we are not going to get a thing done.

DR. GILLETT: It seems that a well baby clinic may be an appropriate analogy. You take a baby to a clinic, not to find out if it is sick, but if it is meeting the standards of the pediatrician for growth, behavior, and performance and is realizing its capacity. We can set similar standards for an ecosystem, even though we can not define an ecosystem exactly. We can look at the components or the processes involving those components. You have to set criteria, as Beverly [Ausmus] and you have been talking about. There is no even measure.

DR. COLE: Why don't we now, for the benefit of the audience, look at some of the pertinent questions. Are we here to study toxic substances, or the environment as a whole, or what? Are microcosms for toxic substances or for anything?

Anybody who wants to answer.

DR. GILLETT: Let's let Dr. Zweig answer that one.

DR. GUNTER ZWEIG: I think you led right into the question I would like to ask each one of the speakers. It seems to me the purpose of this Symposium was to assist the regulatory agencies, and the industries that are to submit their protocols to the Agency for regulatory purposes, in setting up some sort of a protocol and also relate to them the state-of-the-art of microcosms. This morning the microcosms were very simple and well defined. As the afternoon ran on, they became more and more complicated. This colloquium, and the workshop that will follow for some of us, certainly ought to be to tell us what is practical today. I see a few practical things having come out, but I also see a lot of theoretical aspects that will not be ready for a long time. I really would ask each one of the speakers to address themselves to the point: Is your microcosm ready at this moment to assist a regulatory agency in making a decision? Any kind of decision for which any of you have some assistance would help us greatly. This is a self-evaluation, folks.

DR. SYDNEY DRAGGAN: One of the most important things that we have to realize is that if we are doing any kind of chemical hazard evaluation, then we just can not look singly at effects, mobility, or persistence. We have to know about all three to have integrated information. We can have the most toxic chemical that we can think of, but if it doesn't get to the point of action, there is very little hazard to it. Our main objective here is to try to present to you systems that can rapidly give you integrated information on those three, and that is what I hope we have done.

DR. ZWEIG: Can your system be handed to an industry?

DR. DRAGGAN: I don't know if my system could tell you very much about effects. I have seen effects in my system, but I am not sure whether or not I know how to interpret them very well.

DR. ZWEIG: I think the authentic and deeper test is where you can test the effect of a chemical on one or two species, but the system, as has been explained to us by you and the people from Battelle, is so complicated that at this moment I do not think we can cope with it.

DR. DRAGGAN: But we consider it a very simple system.

DR. VAN HOOK: From the scientific point of view as opposed to practical.

AUDIENCE PARTICIPANT: One of the things we have to keep straight with regard to the regulatory concern here is that if anybody is interested in providing a definitive answer as to what happens to a particular chemical when it goes into the environment, then a lot of the approaches that I have heard over the past two days are proper. From a regulatory point of view the best we can hope to do is to determine what the potential, say of the chemical, might be when it is unleashed into an environment and perhaps to test it in what you might call representative systems, whatever they happen to be. If you want to have definitive answers, then you have to test every ecosystem that exists in the whole country, and I do not think that is practical.

The real point is that you have to consider the potential persistence or potential degradation of a particular chemical or particular class of chemicals within the framework of certain types of environmental conditions, and I do not think you can. I agree with Jack [Zweig] that you have to separate out some of these things and then integrate the answers later.

ANOTHER AUDIENCE PARTICIPANT: I am on the other end of this thing. I'm under the gun from EPA to come up with answers on new chemicals. I'm under the gun from our management to come up with the answers on new chemicals. What I really need, if I can be brutally frank about it, is an early warning that is going to tell me, yes, this chemical has a chance of being safe in the environment or, no, this one has some inherent capabilities of damaging the environment and we must reevaluate immediately our expenditure of time, money and effort in further development of this chemical. So if I can back off for just a moment, what looks simple to you is ridiculous to me. When we talk about these ecosystems from the standpoint of industry, we must have a system that gives us a quick answer. I am not quibbling about the work you are doing; frankly I wish I could do some of it myself. We have got to have quick, simple answers that give us a "yes" or a "no," a "go" or a "no-go" decision.

Q: What's the greatest certainty you need?

A: How much profit there is in the chemical.

Q: Say you have a 30 percent chance it is going to be harmful to the environment. Are you going to drop that chemical?

A: Again, how much profit is there in the chemical? As soon as you begin to enlarge my market, then I can take a bigger chance of investing money in the chemical. If I'm going to put out a million tons of this with the 30 percent chance of it being hazardous, that is much different than putting out a few pounds of it in the environment with a 30 percent chance. This is what we would hope to fine in a quick microcosm test system, where we could say very quickly, "This looks too hazardous to pursue it any further; we will drop it at this point and look for another chemical."

DR. VAN HOOK: In response to the last point, in the meetings we have had at the Office of Toxic Substances and a year ago in Annapolis, we looked at microcosms. I thought we still were a part of a tier system of testing, that was not going to give a "yes-no" answer. It may raise a red flag that says you may have a problem in persistence, in magnification, or with regard to a certain kind of an effect. It would never give you a green flag that says this chemical is good. It would just provide a step in your test system and ultimately you are going to go to the field.

AUDIENCE PARTICIPANT: I think you're saying the same thing that I was trying to say. If that red flag is too crucial, we would drop further development at that point.

DR. VAN HOOK: It is conceivable that when you get a red flag, you do some other tests and find out that you can do this, that and the other and get around it. The microcosm test however is not the answer.

DR. COLE: What we would like to get out of the microcosm work that we are doing is to be able to make some kind of evaluation of what is going to happen to a chemical when it gets into the environment and what kind of effects it is going to have there. It boils down to the fact that we really do not understand microcosms and the behavior of materials in them well enough to make that transfer. We can not look at what happens to the material in the microcosm and therefore say what is going to happen to the material in the environment. It seems to me that we need a translatory tool that will give us some way to take microcosm results and say something about the environmental fate and effects therefrom, some kind of quantitative mathematical model. We really do understand a lot about the particular processes that are going on in microcosms, but the complexity of the microcosms that we have worked with has left us with a fuzzy feeling about how they relate to the environment. We ought to be measuring the physiochemical parameters of microcosms and trying to make predictions about what is going to happen to the chemical in the microcosm. Depending on how well we do that job, then some of these other problems we have been concerned about, such as the effects of enclosing the system or the surface-to-volume ratios and so on, would perhaps work themselves out. We could change scales and perhaps even make pretty good predictions of what happens in a large scale environment—a real world system—after having satisfied ourselves that we can do reasonably well with the microcosms. That is the sort of approach and point of view that some of us have taken. Ted Mill, for example, gave a paper that got into that. Hopefully, this comment will stir up someone to say, "No, no, no, you can't do that."

DR. TOM LINDSTROM: As a modeler, thank you very much. That was most encouraging. Apart from what Al [Eschenroeder], Ted [Mill] and a little of what John [Hamaker] said with respect to the quantitation of microcosms and the use of models, very little has been said here. You asked for quick and dirty answers in the sense of signals. The computer can crank out many years worth of material in a few seconds worth of time and at a few dollars cost, if it is provided with the right data, the right estimates of parameters, and the right component parts, i.e. an algorithm. Because the environment is so complex, I do not think we will ever be able to generate a model that will get much better than ball park accuracy, or maybe, if we are lucky with some chemicals, a factor of two. The regulatory people may have to settle for a ball park estimate, if we can be so crude, and that will probably be about as good as we will ever get because the world is just too complicated.

AUDIENCE PARTICIPANT: You have got to convince the judge of that.

DR. LINDSTROM: The point is, a mathematical model or simulator can indeed give you estimates very quickly and somewhat reliably. It is no better than the weakest piece of information you have put in, the weakest physical law, the least assumption that has any validity to it.

DR. ALAN ESCHENROEDER: Of the models that have been used over the past, the one I showed yesterday morning is only one example. In the first place there will never be one grand model. There is no unique model for all compounds.

It depends upon what you want to do and which compound you put in. The rules of the game have been elucidated here very clearly today and yesterday. They change.

Author Biographical Data

MARTIN ALEXANDER, Professor of Agronomy at Cornell University, joined the staff there in 1955, after receiving his M. S. (1953) and Ph.D. degrees in Bacteriology from the University of Wisconsin. He earned his B.S. degree from Rutgers University in 1951. He has chaired and served on many national and international committees involving toxic substances, hazardous materials, soil and plant ecology, and microbiology. As the author of over 200 technical papers and reviews in these areas, he is pre-eminent among soil ecologists and much honored accordingly. He now chairs 2 of the 14 committees, edits or consults on 6 publications, and serves additionally in the Section on Ecology and Systematics, Water Resources Center, and the graduate fields of Ecology, Soil Science, and Microbiology at Cornell.

BEVERLY S. AUSMUS recently joined Battelle-Columbus Laboratories as Principle Research Ecologist after post-doctoral work at Oak Ridge National Laboratory. She obtained her B.S. degree in Biology from Carson-Newman College in 1969 and her M.S. (1971) and Ph.D. degrees in Ecology from the University of Tennessee (1973). She has authored over 50 articles in the areas of microbial ecology and microbial/plant/invertebrate interactions in forest, grassland and desert ecosystems. These studies have involved confined experimental procedures (microcosms) for assessing transport, fate and effects of potentially hazardous heavy metals and other toxic substances.

HAROLD BOND has been recently transferred from the Soil Ecosystem Study Group at the Corvallis ERL to the Ecological Effect Division of the Office of Research and Development, U.S. Environmental Protection Agency. He holds an M.S. in Mycology and Ecology from the University of North Carolina. For the past three years he has been involved in the development of a soil-ecosystem respirator alcrocosm for the evaluation of effects of atmospheric pollutants on decomposition processes. As a U.S. Public Health Service Officer, he has seen service as Chief, Vector Control Studies Laboratory in Mississippi, as a microbiologist with the Great Lakes-Illinois River Basin Project, and as ecologist at the Research Triangle Park Laboratory of USFPA before his Corvallis assignment.

CARY T. CHIOU is a research associate of the Environmental Health Sciences Center of Oregon State University currently involved in studies of the physico-chemical properties of environmentally important chemicals and determination of the relationship of these properties to the movement and persistence of these chemicals in environmental and biological systems. He received his D.S.D. ((1965) in Chemical Engineering at Cheng Kung University (Taiwan) and his M.S. (1970) and Ph.D. (1973) degrees in Physical Chemistry from Kent State University, then carried out post-doctoral work at Brown University and the University of Kentucky before joining the staff of the EHSC in 1975.

LARRY K. COLE served as Research Coordinator with the Toxic Substances Task Force of the Institute of Environmental Studies at the University of Illinois and the Illinois Natural History Survey. He has concentrated his research efforts on the chemistry and environmental toxicology of pesticides and on the development of new insecticides and fungicides. He graduated with a B.A. in Chemistry and Zoology from West Virginia University (1961), received his M.S. in Molecular Biology from the University of Utah in 1968, and earned his Ph.D. in Entomology (pesticides and pheromone toxicology) from the University of Georgia in 1973. He now is a member of the Entomology Faculty at the University of Massachusetts, Amherst.

SIDNEY DRAGGAN has joined the Office of Toxic Substances after 4 years as research ecologist with the Environmental Sciences Division of Oak Ridge National Laboratory. He received his B.S. in the Biological Sciences from City College of New York in 1967, his M.S. in Zoology from Howard University in 1969, and his Ph.D. in Ecology from Rutgers University in 1973. His primary research interests include studies of radionuclide and energy-production related contaminant transport in terrestrial ecosystems which in turn led to the development of microcosms as rapid toxic chemical screening tools for the integrated evaluation of toxic chemical effects, mobility, and persistence in ecosystems.

ALAN ESCHENROEDER received his B.S. and Ph.D. degrees from Cornell University then continued his research on combustion aerodynamics with the U.S. Army Ballistic Research Laboratories. Chemical kinetic and dynamic analyses of hypersonic flows (Cornell Aeronautical Laboratories and GM Defense Research Laboratories) were followed by very successful computer simulation techniques for studying pollutants in the environment at General Research Corp., Santa Barbara. Chemical transformations and physical transport processes are represented in these techniques, which he has extended in environmental modeling work at Environmental Research and Technology, Inc. since 1975.

JAY D. GILE began his research career with the U.S. Environmental Protection Agency in 1971 after completing his M.S. in Marine Biology at Oregon State University. He received his B.S. in Biology and Chemistry from Central Washington State College in 1969. After initial research on radiotracer studies with amphibian and synergism of heavy metal toxicity and radiation to Twinbrook Research Laboratory and Research Triangle Park, he joined the Corvallis ERL, where he has been active in the development and utilization of terrestrial laboratory microcosms.

JAMES WARREN GILLETT is an ecologist and Terrestrial Microcosm Project Leader at the Corvallis Environmental

Research Laboratory, USEPA, and Associate Professor of Agricultural Chemistry at Oregon State University. He received his B.S. in Chemistry (1955) at the University of Kansas and Ph.D. in Biochemistry (1962) from the University of California. After post-doctoral work with Drs. Hoskins and Caside at Berkeley, he continued studies of microsomal induction at Oregon State. His research has focused on toxicological evaluation and modeling of pollutant fate and effects, leading to his joining the USEPA effort in 1974.

JOHN WARREN HAMAKER received his B.A. in Chemistry from U.C.L.A. in 1950, joined the Manhatten Project and received his Ph.D. in Chemistry from the University of California in 1944. After teaching at Napa J.C. and Whittier College in 1954, he joined Dow Chemical Co., where he is a chemist in Agricultural Products Research. His research interests have included ultramicrochemical manipulations, agricultural chelates, physical properties of pesticides, actual and potential behavior of pesticides in soil and the environment, and more recently, the correlation of biological activity and molecular structure of pesticides.

DONALD D. KAUFMAN received his B.S. (1955) and M.A. (1958) degrees from Kent State University and his Ph.D. degree from Ohio State University (1962) in the areas of soil microbiology and biochemistry. He has authored over 100 papers and co-edited 4 books in the areas of pesticide transformations and enzymology, fate and degradation in the environment, microbial ecology and plant pathology. He currently is a Soil Microbiologist at the U.S. Department of Agriculture Agricultural Research Service Pesticide Degradation Laboratory in Beltsville.

E. PAUL LICHTENSTEIN is Professor of Insecticide Toxicology and Entomology at the University of Wisconsin, having received his M.S. and Ph.D. degrees from Hebre University in 1941 and 1948, respectively. He serves on most of the significant national and international committees evaluating and attempting to correct environmental problems caused by pesticides, a role well-justified by his over 100 publications in the area of insect control, ecological factors affecting the fate of insecticides in the environment, biological interactions of pesticides, and naturally occurring toxicants.

WILLIAM J. LISS is a research associate in the Department of Fisheries and Wildlife at Oregon State University, from which he earned his M.S. (1974) and Ph.D. (1977) degrees in Fisheries. His B.S. degree in Zoology was obtained at Pennsylvania State University in 1969. His research interests over the past few years have been theoretical and applied aspects of population and community ecology, theory of exploitation in fish populations, and more recently, conceptual and theoretical constructs for design and evaluation of environmental toxicological studies in laboratory systems.

ROBERT L. METCALF, the "father" of pesticide research in microcosms, has authored perhaps 300 research publications, books, and reviews in the areas of pesticide chemistry and toxicology, insect control, and environmental toxicology. He earned his B.A. (1939) and M.A. (1940) degrees at the University of Illinois and his Ph.D. degree (1943) in Entomology at Cornell University, had a distinguished career at the University of California, Riverside, then returned to his alma mater in 1968 to become Professor of Entomology, Agricultural Entomology, Biology, Veterinary Physiology and Pharmacology, and Research Professor of Environmental Studies. He is Principal Scientist of the Illinois Natural History Survey.

THEODORE MILL is manager of the Physical Organic Chemistry Group at Stanford Research Institute, where he is currently project supervisor for the EPA-sponsored study of the fate of organic compounds in aquatic systems, Principal Investigator for the NSF-RANN study of oxidation mechanisms in aquatic systems, and, with Dr. Mabey, has recently completed a crftical review of the kinetics of hydrolysis of organics in freshwater. Dr. Mill, who received his B.S. in Chemistry in 1953 from Wayne University and his Ph.D. in Chemistry from the University of Washington in 1956, has concentrated his efforts on the persistence of organics in air and water, providing data for laboratory and mathematical simulation and hazard evaluation.

RALPH G. NASH obtained a B.S. degree in Vocational Agriculture, and his M.S. and Ph.D. degrees in Soil Science from Colorado State University. After 2 years with the Central Intelligence Agency as an agricultural analyst, he joined the Pesticide Degradation Laboratory, USDA/ARS, and has published over 50 articles on plant uptake, persistence and movement of pesticides. In 1975 this effort was extended into the microcosm field with the development of the model agroecosystem.

MARVIN E. STEPHENSON is Deputy Director of the Division of Advanced Environmental Research and Technology, NSF-RANN. He received his B.S. from Oregon State University in 1955 and his Ph.D. in Environmental Engineering from the University of California, Berkeley, in 1964. After teaching at the University of Southern California and Michigan State University, and working at the Rockefeller Foundation, he joined the National Science Foundation, where his research interests in physical limnology, physical and chemical separation processes, and environmental science are applied to the fate and effects of organic and heavy metal contaminants in ecosystems.

ROBERT E. VAN HOOK serves as Associate Director of the Energy Development Division at the Oak Ridge National Laboratory. He formerly was project manager of the several successful studies employing microcosm technology and field studies to evaluate the fate and effects of radionuclides and heavy metal contaminents in forest and grassland ecosystems.

JAMES McAULEY WITT is Executive Secretary of the Environmental Health Sciences Center at Oregon State University. After completing his B.S. in Organic Chemistry (1947) at the University of California and his Ph.D. in Agricultural Chemistry, he became Professor of Entomology at the University of Arizona. His research efforts have covered fate and toxicology of pesticides in mammals, crops and the environment. In his current role of Professor of Agricultural Chemistry and Extension Chemist, he addresses a variety of chemical issues for which his advice is sought by industry, environmentalists, and government agencies alike. His recent activities have included a stint with the USEPA attempting to effectuate a Hazard Evaluation System.

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